THE INFLUENCE OF PREVIOUS EXERCISE UPON THE METABOLISM, THE RECTAL TEMPERATURE, AND THE BODY COMPOSITION OF THE RAT [1]

KATHRYN HORST, LAFAYETTE B. MENDEL, AND FRANCIS G. BENEDICT

Nutrition Laboratory, Carnegie Institution of Washington, Boston, Massachusetts, Laboratory of Connecticut Agricultural Experiment Station, and Department of Physiological Chemistry, Yale University, New Haven, Connecticut

(Received for publication May 17, 1933)

Exercise not only causes a marked increase in total metabolism, both during the period of activity and after exercise has ceased, but prolonged, regular exercise alters greatly the chemical composition of the body in that presumably less fat is deposited and hypertrophy of the muscles takes place. These characteristics are accentuated in the trained athlete. Among animals the effects of exercise or lack of exercise are strikingly illustrated by the lean, coursing whippet and the fat, inactive pig. With the rat Asher and Curtis ('25), also v. Árvay and Verzár ('31) have studied the respiratory exchange during a moderate amount of exercise. Our studies dealt with the immediate after-effect of exercise upon the oxygen consumption and the rectal temperature of the rat and also with the effect of prolonged exercise (moderate and severe) upon the metabolism and the body composition.

[1] The data in this paper are taken from a dissertation presented by Kathryn Horst in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Yale University, 1931. The expense of the cooperative research was borne largely by the Nutrition Laboratory of the Carnegie Institution of Washington, Boston. Acknowledgment is also made of assistance from the Connecticut Agricultural Experiment Station and the Russel H. Chittenden Research Fund for Physiological Chemistry.

METHODS

Male albino rats from the Osborne and Mendel colony were used. Some of them were made to run in motor-driven, revolving cages (Reed et al., '30) of sheet metal. In the experiments on the immediate after-effect of exercise the rats (nos. 3, 5, 7, and 9) were exercised 5486 meters daily for about 85 days prior to the special metabolism measurements. In the study of the effect of prolonged muscular activity, growing rats (nos. 1 to 9 and nos. 19 to 27) were exercised daily beginning at the age of 30 days (60 gm.) and continuing until the rats were 130 to 150 days old. The rats exercised between 8.15 A.M. and 12.15 noon and between 2.30 and 5 P.M. In one series the cages revolved twenty-six times, in another eleven times per minute. One revolution equaled 92 cm. An intermittent timer permitted alternately 2 minutes of running and 1 minute of resting. When not running, the exercised rats lived separately in cylindrical metal cages. The control rats were housed separately. The environmental temperature remained at about 25°C. The exercised rats had access to food and water only when not running. Food was not available to the controls when the exercised animals were running.

Experimental feeding began when the rats weighed 60 gm. (30 days old). The following synthetic diet was fed:

Components	*Per cent by weight*
Casein,	18
Salt mixture, Osborne and Mendel ('19),	4
Cornstarch,	54
Butter fat,	9
Lard,	15
Caloric value per gram,[1] 5.2	

This basal diet was supplemented daily with dry brewers' yeast. Rats 1 to 18 also received cod liver oil. The technic of feeding and estimating food consumption was essentially that described by Ferry ('20).

The metabolism was measured with a multiple-chamber respiration apparatus (Benedict, '30). When the rats weighed

[1] The conventional caloric values (protein, 4.1; carbohydrate, 4.1; fat 9.3) were employed.

less than 180 gm. or when short, successive periods were desired (as in measurements immediately after exercise), a small volume of oxygen (about 185 cc.) was introduced into the chamber and the time required for its absorption noted. When the rats weighed 180 gm. or more, the volume introduced was 370 cc. The heat production has been calculated from the measured oxygen consumption (reduced to 0°C., dry, and 760 mm.) on the assumption that the fasting respiratory quotient was 0.72 and that the oxygen consumed had a caloric value of 4.702 calories per liter. The surface area was estimated from the body weight of the rat (in grams) at the close of the metabolism measurement by the formula $S = 9.1 \times w^{2/3}$.

Following the experiments on prolonged exercise, the body composition was determined. Seven rats (approximately 150 days old) and seven controls were killed with illuminating gas after they had fasted (with access to water) 24 hours at 26°C. The fresh weight of the animal, less intestinal contents, was ascertained. The entire animal, including the skin and the intestinal tract (less contents), was put through a food chopper twice. The hashed animal was dried in a partial vacuum at 105°C. After 48 hours of desiccation, the material was ground in a mortar to pulverize pieces of bone. Pieces of skin and hair were cut with scissors. Drying was continued until less than 0.5 gm. of moisture was lost in 12 hours. The moisture content was estimated from the difference in the fresh weight and the weight of the dry tissue. The fat content of the dried tissue was determined by a modification of the Bloor method (Reed et al., '30), the nitrogen by the Kjeldahl method, and the ash by ignition in porcelain crucibles.[3] Each result recorded (table 9) represents the average of at least two well-agreeing determinations.

THE IMMEDIATE AFTER-EFFECT OF EXERCISE UPON METABOLISM

The metabolism of four male rats approximately 125 days old was measured immediately after exercise. On the day

[3] The authors are indebted to Dr. L. L. Reed, of Yale University, for the fat determinations and to Dr. T. G. Phillips and N. F. Colovos, of the University of New Hampshire, for the ash analyses.

preceding the metabolism measurements these rats were not exercised and, to prevent unnecessary drafts upon the body reserves, they had access to food until 24 hours before the metabolism was measured. For 20 hours before the experiment they lived at an environmental temperature of 27°C. After this preliminary treatment, they each ran 4846 meters in 3½ hours (environmental temperature, 25°C.). In order that the metabolism might be determined as soon as possible after cessation of exercise, the respiration apparatus was maintained in a state of equilibrium prior to the moment of beginning the observation on the exercised rat by keeping a 'dummy' rat (really a normal animal of the same weight as the experimental rat) in the respiration chamber for about 1 hour before the experimental animal was to be measured. Fifteen minutes after the dummy rat had been replaced by the experimental animal, measurement of oxygen consumption (at 30°C.) began. Observations were made usually in ten consecutive periods, varying in length from 20 minutes immediately after exercise to 40 minutes several hours later and extending over 5½ hours. The results are recorded in table 1, expressed as cubic centimeters of oxygen (reduced) consumed per 200 gm. of rat per minute. The time after exercise is calculated to the beginning of the particular period of measurement. The first three consecutive low oxygen values for each rat have been italicized. The activity is recorded as the percentage of time that the rat was active during the period of measurement.

The oxygen consumption was higher 15 minutes after exercise than it was later. One hour after exercise it was still from 8 to 10 per cent higher than in subsequent low periods, and a plateau, not necessarily basal, was not reached until 2 to 4 hours after exercise. The sustained after-effect of exercise upon the metabolism of these rats was not unlike that noted with dogs and man after a comparable amount of exercise.

The metabolism of the individual rats varied from period to period. Only one, no. 9 was uniformly quiet during con-

secutive periods of measurement. The metabolism of this rat was 53 per cent (44 per cent, corrected[4]) higher 10 minutes after exercise than in subsequent low periods in the second

TABLE 1

The immediate after-effect of exercise upon the metabolism of the rat (measured at 30°C. and 24 hours after food)

RAT, AGE, AND WEIGHT	TIME AFTER EXERCISE	LENGTH OF PERIOD	O_2 PER 200 GM. PER MINUTE[1]	ACTIVITY	RAT, AGE, AND WEIGHT	TIME AFTER EXERCISE	LENGTH OF PERIOD	O_2 PER 200 GM. PER MINUTE[1]	ACTIVITY
	Hr. Min.	*Min.*	*Cc.*	*Per cent*		*Hr. Min.*	*Min.*	*Cc.*	*Per cent*
No. 9	10	28	4.37	14	No. 5	15	23	4.65	48
137 days	38	35	3.35	6	137 days	38	26	4.12	27
286 gm.	1 13	37	3.17	5	314 gm.	1 04	32	3.34	13
	1 50	41	*2.86*	5		1 36	34	3.14	6
	2 31	41	*2.85*	7		2 10	36	2.96	8
	3 12	41	*2.84*	12		2 46	31	3.44	35
	3 53	40	2.91	12		3 17	34	*3.13*	12
	4 33	37	3.14	16		3 51	34	*3.12*	12
						4 25	37	*2.87*	3
No. 7	21	20	4.63	25					
113 days	41	25	3.80	4	No. 3	13	28	4.05	14
354 gm.	1 06	24	3.95	25	114 days	41	32	3.55	14
	1 30	25	3.79	16	296 gm.	1 13	31	3.66	19
	1 55	23	4.12	35		1 44	32	*3.54*	6
	2 18	26	3.64	4		2 16	34	*3.33*	18
	2 44	24	3.94	25		2 50	35	*3.23*	9
	3 08	25	3.77	16		3 25	30	3.75	23
	3 33	23	4.10	17		3 55	32	3.51	0
	3 56	24	3.92	8		4 27	34	3.31	0
	4 20	26	*3.62*	15		5 01	34	3.22	6
	4 46	28	*3.36*	7					
	5 14	29	*3.16*	10					

[1] Values in italics represent the first three consecutive periods of low oxygen consumption.

and third hours after exercise, 18 per cent higher 38 minutes after exercise, and 11 per cent higher 1 hour and 13 minutes after exercise. The other three rats were occasionally quiet for short periods, but the general picture is one of excessive

[4] Preliminary experiments with rats that had not exercised immediately before metabolism was measured, showed that heat production was from 8 to 9 per cent higher in the first 40 minutes after placing the rat in the respiration chamber than in subsequent periods, even if a state of equilibrium had been reached as explained on page 254.

and variable activity during the first 4 hours after exercise. The restlessness, which was reflected in irregular and increased metabolism, was possibly an after-effect of exercise. It cannot be attributed to the time of day when the metabolism was measured, for it occurred between 11 A.M. and 3 P.M. when rats are usually quiet. Neither can it be attributed to the nature of the rat. When the metabolism of the same animals was measured 40 hours after exercise previous and subsequent to the special measurement immediately after exercise, the rats were seldom active more than 15 per cent of the total 4 hours of observation.

TABLE 2

A comparison of the metabolism of rats 2 to 4 hours after exercise with that 40 hours after exercise (measured at 30°C. and 24 hours after food)

RAT	AGE	BODY WEIGHT	DAYS OF EXERCISE	HOURS AFTER EXERCISE	CALORIES PER SQ.M. PER 24 HOURS	ACTIVITY
	Days	*Gm.*				*Per cent*
No. 9	137	286	97	1¾	*698*	8
	151	302	111	40	744	12
No. 7	113	354	84	4¼	*886*	11
	134	400	105	40	869	6
No. 5	137	314	83	3¼	*767*	9
	156	336	102	40	726	9
No. 3	114	296	78	1¾	*833*	11
	128	320	92	40	774	18

The average heat production of each rat during the first three consecutive low periods immediately after exercise (italicized in table 1) is recorded in italics in table 2. The heat production of each of the same rats measured 40 hours after exercise at a date 3 weeks after the special measurement is also shown in table 2. At this time the rats had exercised for about 100 days. In an attempt to eliminate, in so far as possible, the effect of the differences in weight of each rat between the time of the first and the second metabolism observation reported in table 2, the heat production in each

case is expressed per square meter of body surface. Comparison of the two observations on each rat shows that the metabolism of rat 9 was lower 1¾ hours after exercise than it was in the subsequent measurement 40 hours after exercise. That of rat 7 was not much higher 4¼ hours after exercise than it was 3 weeks later, 40 hours after exercise. That of rat 5 was 6 per cent higher 3¼ hours after exercise and that of rat 3 was 8 per cent higher 1¾ hours after exercise than in the subsequent observations 40 hours after exercise. Moreover, the metabolism of rat 3 was not much lower in the fourth and fifth hours after exercise (table 1). Hence, although the metabolism of these rats reached a plateau in from 2 to 4 hours after exercise, it was in at least two instances from 6 to 8 per cent above the basal level. With rats forced to run 440 meters per hour for 2 hours or about one-third the hourly speed of our exercised rats, v. Árvay and Verzár ('31) noted that the metabolism was high immediately after work and decreased continually until the third half-hour after exercise, when it reached a normal level. The metabolism of our rats that ran at a faster rate for a longer period did not reach a normal level by the third half-hour.

THE AFTER-EFFECT OF EXERCISE UPON RECTAL TEMPERATURE

The marked effect of muscular exercise upon human body functions and the return of these functions to normal after having been affected by exercise have been carefully studied by H. M. Smith ('22). After severe grade walking indications were found of a rapid rise in rectal temperature and an equally rapid fall. The technic of rectal temperature determinations on rats as described in a previous communication (Benedict et al., '32) has been satisfactory. Hence as a part of our study of the immediate after-effect of exercise upon the rat, observations of the rectal temperature were included. Nine male rats were measured that had exercised approximately 65 days and, to study the normal daily rhythm of the body temperature of the non-exercised rat, nine littermate controls were also measured at three different times of day.

The rectal temperatures of the exercised rats were measured before work (8.30 to 9 A.M.), immediately after the rats had run continuously for 4 hours (5486 meters), and thereafter at intervals of 20 minutes for 85 minutes. These measurements were made on three different days at weekly intervals. The results of a typical day alone (average age of rats, 100 days) are presented in table 3.

The rectal temperatures before exercise varied only from 37.1° (rat 5) to 37.9°C. (rats 3 and 7). Although it is debatable whether one should average values for only nine rats,

TABLE 3

Rectal temperatures of rats before and after exercise

RAT NO.	BEFORE EXERCISE (8.30 A.M.)	AFTER EXERCISE [1] (12.30 TO 2.00 P.M.)				
		0 minute	20 minutes	40 minutes	60 minutes	85 minutes
	°C.	°C.	°C.	°C.	°C.	°C.
1	37.4	39.3	38.2	38.1	37.8	37.7
2	37.3	38.9	38.3	38.6	38.2	37.4
3	37.9	39.2	38.8	37.2	39.1	37.6
4	37.7	39.3	38.2	37.8	37.6	37.6
5	37.1	38.3	37.9	37.4	37.3	37.7
6	37.2	38.9	37.7	37.3	37.0	37.1
7	37.9	39.2	38.8	38.8	38.4	38.1
8	37.2	39.2	38.3	37.8	37.6	37.6
9	37.6	38.4	38.2	37.8	37.7	37.7
Average	37.5	39.0	38.3	37.9	37.9	37.6

[1] After rats had been running 4 hours (5486 meters).

the uniformity in the results is such that the average value of 37.5° may be accepted as representing with reasonable accuracy the pre-work level. Immediately after exercise the temperatures averaged 39.0°, i.e., 1.5° above the pre-work level. Twenty minutes later the temperatures had fallen to an average of 38.3° and in another 20 minutes to 37.9°, at which level they remained essentially constant for the remainder of the time. Occasionally an aberrant value appears, such as the observed temperature of rat 3 (39.1°) at the 60-minute period, which was nearly 2° above the preceding value (37.2°) at the 40-minute period. This rat struggled,

however, during the measurement at the 60-minute interval. The observed rectal temperatures after exercise did not actually reach the pre-work level, but at the end of 85 minutes they were on the average only one-tenth of a degree above the pre-work values.

The measurements before exercise may not, however, be accepted uncritically as the baseline. They were made at 8.30 A.M., 4 hours before the next determinations at 12.30 P.M.

TABLE 4

Daily rhythm of rectal temperatures of non-exercised rats

RAT 10	RAT 11	RAT 12	RAT 13	RAT 14	RAT 15	RAT 16	RAT 17	RAT 18	GROUP AVERAGE
°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Morning[1]									
37.2	37.9	37.6	37.6	37.8	37.4	37.6	36.6	37.4	
37.3	38.0	36.9	37.6	37.6	37.7	37.4	36.9	37.4	
37.3	37.2	37.0	37.2	37.4	38.6	37.0	36.7	37.2	
37.1	38.1	37.0	37.4	37.8	38.0	37.4	37.4	37.6	
37.2	37.8	37.1	37.5	37.7	37.9	37.4	36.9	37.4	37.4
Noon[1]									
37.6	37.9	36.8	37.6	38.0	37.7	37.1	37.3	37.4	
37.2	37.9	37.2	37.7	37.3	37.4	37.4	36.9	37.7	
37.4	37.4	37.0	37.8	37.9	37.6	37.4	37.0	37.6	
37.7	37.6	37.2	37.0	37.6	37.3	37.3	37.3	37.7	
37.5	37.7	37.1	37.5	37.7	37.5	37.3	37.1	37.6	37.4
Evening[1]									
37.3	38.6	37.9	37.0	37.7	38.6	37.4	38.1	38.2	37.9

[1] 9.00 A.M.; 12.00 noon; 6.00 P.M.

immediately after exercise. Hence, if a diurnal rhythm existed, it would appear here. To secure information on any possible diurnal variation in body temperature, control rats (table 4) were measured on 4 different days at 9 A.M. and 12 noon, and on 1 day at 6 P.M. These rats engaged only in spontaneous cage activity and were fairly quiet during the day. Innumerable temperature measurements have already been made upon the normal rat, but the values in table 4 have special interest, since they indicate the rectal tempera-

ture at definite times throughout the day. In general, the values for the individual animals agree well. In the morning the variation in the rectal temperatures of any one rat on the 4 different days was frequently not more than 0.4°C. Rats 11, 15, and 17, however, showed differences of not far from 0.9°C. The average morning temperatures of the several rats on the 4 days differ only by 1.0°C. In the noon series the differences between determinations on the same animal on different days was usually less than in the morning series. Only rats 13 and 14 showed a variation of 0.7° or over in the four measurements. The 4-day noon averages for the various animals agree more closely than the morning averages, differing only by 0.6°C. The greater uniformity is undoubtedly a reflection of the greater degree of repose at midday. In the one series of measurements at 6 P.M. there was a variation of 1.6°C. in the values. The average evening temperature for the group (37.9°) is somewhat higher than the group average at noon (37.4°). Thus the rats had a more uniform rectal temperature when in greatest repose, whereas with probable differences in activity, even only cage activity, they showed greater differences in body temperature. These differences in man would represent an actually febrile state. Judging from our noon series, however, we can assume that the average rectal temperature of the normal resting albino rat at midday is close to 37.4°C.

The rectal temperatures of the exercised rats before work at 8.30 A.M. averaged 37.5° (table 3); immediately after work at noon, 39.0°; and from 40 to 85 minutes later, 37.9°C. The rectal temperatures of the non-exercised animals averaged 37.4° at 9 A.M. and again at noon. Hence the high temperatures of the exercised rats immediately after exercise at noon cannot be attributed to a diurnal rhythm but must have been an after-effect of the exercise.

THE EFFECT OF PROLONGED MUSCULAR EXERCISE UPON BASAL METABOLISM

Two series of experiments were conducted to determine the effect of prolonged muscular activity upon basal metabolism. In both series the exercised rats were forced to run about 548 meters daily when exercise was initiated. During the first 2 weeks the distance run was gradually increased until the rats in the first series ran 5486 meters daily (table 5) and those in the second series, 1829 meters daily (table 6). The metabolism of these exercised rats was measured several times between the ages of 35 and 156 days, during which time their body weights naturally increased rapidly. Littermates were selected for controls, since our experience has been that the metabolism of littermates is usually in close agreement.[5] The moderately exercised rats and their littermate controls were fed the basal ration ad libitum and from 200 to 250 mg. of yeast daily. The severely exercised rats (nos. 1 to 9) were given the basal ration ad libitum and 600 mg. of yeast daily; some of their littermate controls (nos. 10 to 14) also received an unlimited basal ration and from 200 to 400 mg. of yeast daily, and others (nos. 15 to 18) were given a restricted basal ration and 600 mg. of yeast daily. These two methods of feeding were employed in an attempt to maintain the rate of growth of the controls at a rate comparable to that of the exercised rats. The controls were not exercised but were permitted the cage activity normal for a rat living alone. For 24 hours prior to the metabolism measurements the rats lived in a special cage (Benedict and Riddle, '29) at a known and constant environmental temperature of 25° or 28°C. After a 1-hour preliminary period of adjustment to the experimental conditions, the time required for each rat to absorb a known volume of oxygen was determined. Three or more consecutive periods, circa 50 to 100 minutes long, were carried out between 10 A.M. and 4 P.M.

[5] Greenman and Duhring ('31) state that animals from the same litter are more nearly alike than others of the same age.

Since in all our experiments exercise was initiated when the rats were about 30 days old, the factor of growth or advancing age undoubtedly enters into this study. Likewise, it is conceivable that the effect of muscular activity may be more pronounced in the young growing rat than in the same animal when full grown. The effect may also be more pronounced early in the period of training. Another point to be considered is that, although littermates were selected for controls, thus ruling out the influence of difference in age in the different age groups, the attempt to feed the control animals so that their weights would be the same as those of the exercised rats was only partly successful in the case of the first series (severe exercise). As will be seen from analysis of the data in table 5, the metabolism of the controls fed restricted rations was somewhat lower (at all the different ages) than that of the controls on full rations. Hence, the average values reported in table 5 for the controls are somewhat lower than they would be had all the control rats been on full rations. Still another point to be held in mind is that the control rats were not wholly inactive. How active they were is not known. Moreover, although the distance traveled by the exercised rats was ascertained, this distance does not represent a quantitative estimate of the amount of work done. The rats could not cling to the sides of the cage and be carried around by mechanical action, but they might have slipped and rolled inside the cage.

To eliminate, in so far as possible, the effect of differences in weight, investigators have commonly expressed the metabolism in terms of the heat production per square meter of body surface. In order to present our data in a form comparable to that employed by earlier investigators and pending a future calculation on some other basis, we have referred the heat production of our rats in tables 5 and 6 to the body surface. Our moderately exercised rats weighed essentially the same as their controls. Likewise, in the severe exercise series the weights of the controls themselves were much the same in any given age group. Hence, we have also reported

TABLE 5

Effect of prolonged, severe (5486 meters per day) exercise upon the basal metabolism of the growing male rat (measured 24 hours after food, 40 hours after exercise, and at 30°C.)

EXERCISED RATS					NON-EXERCISED RATS			
Rat no.	Age	Weight	Days of exercise	Heat production per sq.m. per 24 hours	Rat no.	Age	Weight	Heat production per sq.m. per 24 hours
	Days	*Gm.*		*Cal.*		*Days*	*Gm.*	*Cal.*
1	48	95	18	947	10	48	94	888
2	65	132	25	886[1]	11	48	97	828
3	51	104	15	930	12	51	87	838
4	65	115	25	1000	13	62	100	852
5	67	118	13	913	15	43	99	754
6	62	115	22	940	16	51	86	831
7	43	107	14	985	17	62	111	838
8	56	99	18	908	Aver.	52	96	833
9	65	119	25	900				
Aver.	58	112	19	934				
2	86	188	46	912	14	72	123	960[1]
1	76	197	46	1062	10	76	171	832
3	72	175	36	944	11	76	171	835
4	93	180	53	773[1]	13	93	172	874[1]
5	88	182	34	859	18	76	158	723
6	93	187	53	822[1]	15	76	167	739
7	83	274	54	876	16	79	167	824
8	79	187	41	891	17	88	161	783
9	88	182	48	814	Aver.	80	161	821
Aver.	84	195	46	884				
1	99	240	69	979	11	99	238	901[1]
3	91	249	55	870	10	99	233	843
2	114	247	74	809	12	121	293	818
4	121	237	81	782	14	121	233	807
5	109	255	55	845	13	121	238	896[1]
6	114	240	74	866	18	99	232	819[1]
8	121	284	83	755	16	121	285	779
9	114	255	74	782[1]	17	121	247	736[1]
Aver.	110	251	71	836	Aver.	113	250	825
1	132	308	102	768	10	132	298	784
3	128	320	92	774[1]	12	142	328	691
4	151	292	111	740	13	142	279	716
5	156	336	102	726	18	134	296	738
6	142	276	102	758	15	141	352	645
2	156	290	116	773[1]	17	151	302	747
7	148	410	119	818	Aver.	140	309	720
9	151	302	111	744				
Aver.	146	317	107	763				

[1] Rats were active from 15 to 20 per cent of the period of measurement; activity was less than 15 per cent for values not marked.

TABLE 6

Effect of prolonged, moderate (1829 meters per day) exercise upon the basal metabolism of the growing male rat (measured 24 hours after food, 40 hours after exercise, and at 30°C.)

EXERCISED RATS					NON-EXERCISED RATS			
Rat no.	Age	Weight	Days of exercise	Heat production per sq.m. per 24 hours	Rat no.	Age	Weight	Heat production per sq.m. per 24 hours
	Days	*Gm.*		*Cal.*		*Days*	*Gm.*	*Cal.*
19	44	94	14	856	28	44	96	916
20	45	87	14	749	29	45	98	798
21	42	98	12	803	30	42	88	822
22	35	67	8	847	31	35	74	763
23	42	70	6	813	32	42	77	800
24	44	84	14	777	33	44	80	899
25	42	74	6	831	Aver.	42	86	833
26	42	72	6	810				
27	35	70	8	781				
Aver.	41	80	10	807				
					28	60	138	909
19	60	132	30	903	29	64	134	727
20	64	134	33	761	30	58	130	906[1]
21	58	139	28	869	31	56	113	746
22	56	104	29	861	32	57	102	834
23	57	102	21	784	Aver.	59	123	824
24	70	145	40	681				
25	57	91	21	848				
26	57	100	21	806				
27	56	113	29	812				
Aver.	59	118	28	814	30	84	194	990[1]
					31	75	160	713
21	86	190	56	807	32	76	141	915[1]
22	75	148	48	835	Aver.	78	165	873
23	76	144	40	896				
25	76	149	40	891				
26	76	141	40	874[1]				
27	84	154	57	816	28	88	189	840[1]
Aver.	79	154	47	853	29	94	188	763
					Aver.	91	189	802
19	93	191	63	954				
20	99	190	68	748				
23	99	178	63	872				
24	91	186	61	754				
25	97	193	61	855	28	123	230	611
26	99	176	63	899	29	133	236	713
Aver.	96	186	63	847	30	114	234	815
					31	131	236	724[1]
20	133	229	102	804	32	134	224	851
21	114	234	84	783	Aver.	127	232	743
22	131	228	104	877				
23	132	232	96	860[1]				
24	128	244	98	696				
25	132	251	96	881				
26	139	219	103	870[1]				
27	131	239	104	980[1]				
Aver.	130	235	98	844				

[1] Rats were active from 15 to 20 per cent of the period of measurement; activity was less than 15 per cent for values not marked.

the basal metabolism of each age group in both series on the basis of the average heat production per 200 gm. of body weight per 24 hours (table 7).

The metabolism of the severely exercised rats and their controls (table 5), both per square meter of body surface and per 200 gm. of body weight decreased, on the average, with age. On the contrary, the metabolism of the moderately exercised rats (table 6) per unit of surface area did not decrease with age. Per unit of weight, however, the metabolism of the moderately exercised rats did show the characteristic

TABLE 7

Average heat production per 200 gm. of body weight per 24 hours of exercised rats and their controls

EXERCISED RATS				CONTROLS		
Distance run daily	Average age	Average weight	Calories per 200 gm. per 24 hours	Average age	Average weight	Calories per 200 gm. per 24 hours
Meters	*Days*	*Gm.*		*Days*	*Gm.*	
5486	58	112	35.5	52	96	33.1
5486	84	195	27.9	80	161	27.6
5486	110	251	24.1	113	250	23.9
5486	146	317	20.4	140	309	19.4
1829	41	80	34.3	42	86	34.5
1829	59	118	30.4	59	123	30.2
1829	79	154	29.1	78	165	29.0
1829	96	186	27.1	91	189	25.5
1829	130	235	24.9	127	232	22.0

decrease with advancing age and increasing weight. The controls for this series showed a lower metabolism with advancing age on both bases of calculation.

On the body-surface basis the average heat production of the severely exercised rats at an average age of 58 days was 12 per cent higher than the average for their littermate controls, and at 84 days of age it was 8 per cent higher. At 110 days and 146 days it was still somewhat higher than that of the controls, but not significantly higher. Indeed, at 146 days the heat production of both the severely exercised and the control rats approached the average metabolism, 708

calories, reported previously (Benedict, Horst and Mendel, '32) for adult male rats weighing from 300 to 400 gm. On the body weight basis the average metabolism of the severely exercised rats in the several age groups was not appreciably higher than the average metabolism of their controls.

The average heat production of the moderately exercised rats was almost the same per unit of surface area at 41 days of age, with 1 or 2 weeks of previous exercise, as at 59 days of age, after 3 to 4 weeks of exercise. Likewise, the average metabolism was essentially the same as that of the littermate controls at these ages. It is possible that the exercised rats were quiet when not forced to run and that the spontaneous activity (not recorded) of the caged controls throughout the night was so great that in the end the exercise of the animals in the two groups was about the same. It is also possible that the exercised rats did not receive sufficient vitamin B. Initially they were given not much more than 200 mg. of dry brewers' yeast daily. Keith and Mitchell ('23) concluded that forced running (about 3 miles a day) did not clearly affect the rat's requirement for vitamin B. More recently, however, Cowgill, Rosenberg, and Rogoff ('31) have shown that in the dog the time required to deplete the vitamin B reserve is decreased by exercise. At 79 days the moderately exercised rats had again much the same average metabolism per square meter of body surface as their controls, but at 96 and 130 days their metabolism on this basis was higher than that of the controls. On the body weight basis the moderately exercised rats had a heat production averaging essentially the same as that of their controls.

If one were to consider only the heat production of the severely exercised rats and their controls on the basis of per unit of surface area, one could conclude that the severe exercise (although the exercise had ceased 40 hours before the metabolism was measured) increased the basal metabolism of the rats in the growing stage, but that when the adult weight (about 300 gm.) was reached, the severely exercised rats had a metabolism typical of the normal non-exercised

rat. Against such a conclusion, however, is the fact that per unit of body weight the metabolism of the severely exercised rats was much the same as that of their controls (especially if one considers only the controls on full feed) and that on both bases of calculation the metabolism of the moderately exercised rats was essentially the same as that of their controls, at all ages. Since the controls for the moderately exercised rats were more ideal for comparison, being nearly of the same weights as the exercised rats, it seems reasonable to conclude that continued, forced running amounting to at least 1829 meters and probably to 5486 meters daily has no influence on the basal metabolism of the rat.

This finding is difficult to interpret, since one would expect that prolonged exercise would cause a pronounced alteration in the composition of the body (less deposition of fatty tissue) and that the hypertrophy of protoplasmic tissue, particularly muscle tissue, would result in a greater cell activity and hence an increased metabolism. This would be in harmony with the general view that athletically trained individuals have a high metabolism. This last point, however, is justly debatable. Benedict and Smith ('15) considered that the athletes studied by Smith at Syracuse University had a metabolism somewhat higher than normal. In a subsequent recalculation of these values, Harris and Benedict ('19) decided the difference was not so great as had previously been estimated. Schneider and his associates at Wesleyan University ('27, '31) found that in some instances the metabolism of human subjects was lowered by exercise, in others increased, and in others there was no change. It is a question whether in the cases mentioned the men were given proportionately the same amount of exercise that our rats were given. The exercise of man is usually maintained only during a relatively short time, whereas our rats ran for many hours daily. Hence the exercise entered as a larger factor in the case of our rats than in either of the cases cited for man.

The contradictory results shown by the heat values (per unit of body surface and of body weight) of our severely exercised

rats are undoubtedly ascribable to the fact that the controls used for comparison were not of the same weight as the exercised rats. This was a defect in our experimental technic. It also accentuates the chaotic state existing at the present day in the methods employed in comparing the metabolism of animals of different sizes, even of the same species, and especially challenges the infallibility of the surface-area comparison. Obviously, in any further experiments where the effect upon the basal metabolism of some superimposed factor is to be studied, the control animals must be of the same age and weight throughout the entire period of comparison, no matter what experimental difficulties are entailed in the securing of such controls.

RELATION OF FOOD CONSUMPTION TO SEVERE EXERCISE OF GROWING RATS

The food consumed by the severely exercised and by the control rats during the experimental period of about 120 days is recorded in table 8, expressed as the average daily food intake of each rat. The relation of food consumption to increment in weight is also shown. Since the food was essentially water-free and compounded so that 1 gm. had a caloric value of about 5 calories, we can deal directly with the relative weights of food consumed. Per gram of gain in weight the exercised rats consumed more food than the controls, 5.1 as compared with 4.3 gm. The caloric intake was thus about 26 calories per gram of gain in weight for the exercised rats and 22 calories for the controls. The total daily food intake of the exercised rats averaged 11.3 gm. and that of the controls, 9.1 gm. The exercised rats therefore consumed daily 2.2 gm. or about 24 per cent more than did the non-exercised rats. Since both groups started at the same weight (about 60 gm.) and ended at approximately the same weight (about 300 gm), one might infer that the muscular work of the exercised rats raised the metabolism only approximately 24 per cent, that is, in proportion to the increase in food consumption. In any comparison of the food intake

with gain in weight from the energetic standpoint, however, one must always realize that there is a large element of uncertainty with regard to the normal energy requirements of the animals when not exercising. In analyzing cases like these the old, classic method of Rubner of determining the total metabolism throughout 24 hours would have been highly

TABLE 8

Relation of severe exercise (5486 meters daily) to food consumption of growing male rats

RAT NO.[1]	DURATION OF EXPERIMENT	BODY WEIGHT			FOOD INTAKE	
		Initial	Final	Average daily gain	Average per day	Per gram gain in weight
	Days	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Exercised						
1	110	58	309	2.3	10.7	4.7
2	126	61	286	1.8	10.8	6.1
3	115	70	339	2.3	11.6	5.0
5	128	60	344	2.1	10.8	5.0
7	133	70	427	2.7	13.7	5.1
8	115	51	316	2.3	10.9	4.7
9	126	60	312	2.0	10.7	5.4
Average					11.3	5.1
Controls						
10	121	62	305	2.0	9.9	4.9
12	122	56	324	2.2	10.0	4.5
13	133	63	316	1.9	9.1	4.7
15[2]	134	68	381	2.3	8.9	3.8
16[2]	123	58	327	2.2	8.7	4.0
17[2]	134	67	336	2.0	8.8	4.4
18[2]	121	50	303	2.1	8.6	4.1
Average					9.1	4.3

[1] Rats lived at 25°C.

[2] Rate of growth controlled by limiting food consumed.

successful. Our complete lack of knowledge of the metabolism of these rats, not under basal conditions, but while resting or at least while not forcibly exercised, complicates the situation enormously. Measurement of the metabolism in a calorimeter, where the rats would be allowed normal, free activity as compared with the forced activity in the exercise chamber, would have solved the problem.

It is inconceivable, however, that the severe exercise (5486 meters daily) increased the metabolism only 24 per cent, unless the spontaneous activity of the controls was nearly the same as the forced exercise of the experimental rats, under which conditions the difference in energy consumption would represent the true difference in heat production. This brings out strikingly the difficulty of analyzing food intake in terms of energy, especially where there is a possibility that the superimposed factor of exercise may have altered the body composition. It is logical to consider the food intake from the standpoint of calories, but it is not correct to consider the increase in an animal's weight from the energy standpoint. In such a consideration one assumes that material of the same composition and hence of the same energy value was added to the body weight throughout the entire time. However, there may have been a deposition of fat and a compensating loss of water, which would alter the energy value of the body material. It is perhaps surprising that the exercised rats did not eat considerably more than the controls. We have no knowledge of the actual distance covered by the controls. They were allowed 'cage activity,' but we do not know to what extent they roamed around their cages at night. On the other hand, we also do not know how quiet, relatively, the exercised rats were at night following their severe exercise during the day. We might fairly assume that there was a compensation in activity and that the exercised rats did not travel around as much at night as did the controls.

THE BODY COMPOSITION

The idea that severe, long-continued exercise will alter the deposition of fatty tissue and possibly the transport of water or the variation of water content is not new. Reed, Yamaguchi, Anderson, and Mendel ('30) found that exercised rats deposited less fat than non-exercised rats. Variations in body composition have also been cited to account for differences in metabolism. In general, the bodies of our severely exercised rats (5486 meters daily) were 3 per cent richer in water

than the bodies of the controls and consequently contained a lower percentage of dry matter (table 9). On the basis of fresh weight the bodies of the exercised rats contained, on the average, 3.5 per cent less fat, 0.35 per cent more ash, and 0.06 per cent more nitrogen than those of the controls. On the basis of dry matter the exercised rats had a higher ash content than the controls, the difference amounting to 1.58 per cent. The average nitrogen content was 0.78 per cent higher in the exercised rats than in the controls. However, some rats in both the exercised and the control groups showed high nitrogen percentages. For example, rat 1 (exercised) had a nitrogen content of 10.25 per cent and rat 16 (not exercised) of 10.13 per cent. These two rats had the lowest dry weights and the lowest fat percentages of all the animals in both groups. On the dry basis the average fat content of the exercised rats was 6.4 per cent lower than that of the controls. The range in the fat content on the dry basis was greater in the controls than in the exercised animals. The percentage of residual protoplasmic matter, devoid of extraneous components, was apparently constant so far as indicated by the nitrogen content. Thus the percentage of nitrogen in the moisture, fat, and ash-free tissue averaged 16.4 for both groups of rats.

In general the influence of prolonged severe exercise was to raise the water content and lower the fat content of the body. Of the dry matter remaining, the ash content was higher, the nitrogen content higher, and the fat content lower than in the controls. Aside from the high percentage of ash noted with rat 1, no other striking changes in the body composition of the exercised rats were apparent. The heat production was in no way related to the ash content. The heat production singularly enough had no direct relationship to the nitrogen content. To be sure, control rats 15 and 12 with the lowest heat production also had the lowest nitrogen content. On the other hand, control rat 10 with the highest metabolism had a medium nitrogen content, and exercised rats 8, 9, and 1 with heat values somewhat lower than that

TABLE 9

The influence of prolonged severe exercise upon the body consumption of male rats

RAT NO.	DURATION OF EXPERIMENT	FRESH WEIGHT	DRY WEIGHT	PER CENT OF SOLIDS	FRESH WEIGHT				DRY WEIGHT			PER CENT OF NITROGEN MOISTURE-ASH FAT-FREE
					Moisture	Fat	Nitrogen	Ash	Fat	Nitrogen	Ash	
	Days	*Gm.*	*Gm.*		*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	
Exercised												
1	111	309	96	31.1	68.9	6.7	3.19	3.78	21.5	10.25	12.17	15.5
2	127	286	105	36.7	63.3	13.7	3.18	3.55	37.0	8.66	9.67	16.2
3	116	339	126	37.2	62.8	14.8	3.10	3.45	40.0	8.33	9.27	16.4
5	130	344	129	37.5	62.5	15.4	3.09	3.51	41.1	8.25	9.35	16.7
7	134	427	152	35.6	64.4	13.5	3.12	3.24	37.9	8.76	9.11	16.6
8	116	316	114	36.1	63.9	13.8	3.39	3.39	38.4	9.40	9.40	18.0
9	127	312	114	36.5	63.5	14.2	2.94	3.41	38.8	8.05	9.34	15.5
Average				35.8	64.2	13.2	3.14	3.48	36.4	8.81	9.76	16.4
Controls												
10	122	305	114	37.4	62.6	15.6	3.07	3.48	41.6	8.22	9.31	16.7
12	123	324	126	38.9	61.1	17.5	2.86	3.24	44.8	7.36	8.33	15.7
13	134	316	123	38.9	61.1	16.0	3.21	3.25	41.2	8.25	8.35	16.4
15[1]	135	381	160	42.0	58.0	22.7	3.04	2.93	53.8	7.23	6.98	18.4
16[1]	123	327	109	33.3	66.7	9.8	3.37	3.16	29.4	10.13	9.49	16.6
17[1]	134	336	133	39.6	60.4	16.9	3.03	2.83	42.8	7.64	7.15	15.3
18[1]	122	303	121	39.9	60.1	18.2	2.95	3.05	45.7	7.40	7.64	15.9
				38.6	61.4	16.7	3.08	3.13	42.8	8.03	8.18	16.4

[1] Rate of growth controlled by limiting food consumed.

of rat 10 had higher nitrogen contents. There was also no direct relationship between the basal metabolism and the fat content. In the exercise series the lowest heat production was observed on rat 5, which had the highest fat content of any of the rats in this group. But rat 7 with the highest metabolism had a medium fat content and rat 1 with an intermediate metabolism had a low fat content. In the control series rat 15 with the lowest metabolism had the highest fat content and rat 16 with a relatively high metabolism had a low fat content, but rat 10, also with a high metabolism, had a medium fat content. From these data, on the whole, no real difference in metabolism is ascribable to variations in fat content.

The metabolism of our controls was lower and the fat content higher than the values reported by Brendle ('31) for rats of the same size and sex. This comparison supports Brendle's conclusion that the metabolism is lower with a higher percentage of fat. It is debatable, however, whether metabolism measurements as short as 10 minutes are justifiable. Since only 10-minute periods were used by Brendle and his averages are based on periods with greatest repose, one would expect his values in these selected short periods to be even lower than those in our experiments in which the metabolism was measured during at least 2 or 3 hours (three consecutive periods). Another fact to be considered is that our exercised rats had nearly 4 per cent less fat than our controls and yet their metabolism was approximately the same.

SUMMARY

The oxygen consumption (at 30°C. and 24 hours after food) of four male rats was high immediately after exercise, and 1 hour after exercise it was still from 8 to 10 per cent above subsequent low periods. In from 2 to 4 hours after exercise the metabolism reached a plateau that was approximately basal for two of the rats but still from 6 to 8 per cent above basal for the other two rats.

The average rectal temperature of nine male rats after exercise (39.0°) was 1.5° higher than the pre-work level (37.5°C.). During the first 40 minutes after exercise the temperatures fell rapidly and during the next 45 minutes remained essentially constant at a level, on the average, from 0.1° to 0.4°C. above the pre-work values. The average rectal temperature of non-exercised rats at noon was the same as in the morning, 37.4°C.

Prolonged severe exercise (5486 meters daily) increased the basal heat production per square meter of body surface (measured 40 hours after exercise) only of growing male rats, but not of adult rats. Per unit of body weight, on the contrary, the metabolism of the exercised rats was much the same as that of their littermate controls, both during growth and during adult life. The picture is not clear because the controls were not of the same body weights as the exercised rats and were fed somewhat differently.

Prolonged moderate exercise (1829 meters daily) did not modify the basal metabolism of the growing and adult male rats, either per unit of body weight or per unit of body surface. In this study the littermate controls were fed exactly the same as the exercised rats and weighed approximately the same.

The basal metabolism of both groups of exercised rats and of the controls decreased with advancing age.

The severely exercised rats (5486 meters daily for 120 days) consumed only about 24 per cent more food than the non-exercised rats. On the basis of fresh weight the bodies of the exercised rats contained 3.5 per cent less fat and 3 per cent more water than the bodies of the non-exercised rats. On the dry basis the exercised rats had an ash content 1.58 per cent higher, a nitrogen content 0.78 per cent higher, and a fat content 6.4 per cent lower, on the average, than the non-exercised rats. Variations in basal metabolism were not directly related to differences in ash, nitrogen, and fat content.

LITERATURE CITED

v. ÁRVAY, A., AND F. VERZÁR 1931 Der Gaswechsel bei Muskelarbeit nach Entfernung der Nebennieren. (Versuche an Ratten.) Biochem. Zeitschr., Bd. 234, S. 186.

ASHER, L., AND G. M. CURTIS 1925 Beiträge zur Physiologie der Drüsen. Untersuchungen über den respiratorischen Stoffwechsel bei Arbeit in seiner Beziehung zu den Drüsen mit innerer Sekretion. Biochem. Zeitschr., Bd. 164, S. 97.

BENEDICT, F. G. 1930 A multiple-chamber respiration apparatus for rats and other small animals. J. Nutrition, vol. 3, p. 161.

BENEDICT, F. G., K. HORST, AND L. B. MENDEL 1932 The heat production of unusually large rats during prolonged fasting. J. Nutrition, vol. 5, p. 581.

BENEDICT, F. G., AND O. RIDDLE 1929 The measurement of the basal heat production of pigeons. I. Instrumental technique. J. Nutrition, vol. 1, p. 475.

BENEDICT, F. G., AND H. M. SMITH 1915 The metabolism of athletes as compared with normal individuals of similar height and weight. J. Biol. Chem., vol. 20, p. 243.

BRENDLE, E. 1931 Gaswechsel und Körperzusammensetzung. Arch. f. d. ges. Physiol., Bd. 226, S. 108.

COWGILL, G. R., H. A. ROSENBERG, AND J. ROGOFF 1931 Studies in the physiology of vitamins. XVI. The effect of exercise on the time required for the development of the anorexia characteristic of lack of undifferentiated vitamin B. Am. J. Physiol., vol. 98, p. 589.

FERRY, E. L. 1920 Nutrition experiments with rats. A description of methods and technic. J. Lab. and Clin. Med., vol. 5, p. 735.

GREENMAN, M. J., AND F. L. DUHRING 1931 Breeding and care of the albino rat for research purposes. Philadelphia, 2nd ed., p. 12.

HARRIS, J. A., AND F. G. BENEDICT 1919 A biometric study of basal metabolism in man. Carnegie Inst. Wash. Pub. no. 279, p. 244.

KEITH, M. H., AND H. H. MITCHELL 1923 The effect of exercise on vitamin requirements. Am. J. Physiol., vol. 65, p. 128.

OSBORNE, T. B., AND L. B. MENDEL 1919 The nutritive value of the wheat kernel and its milling products. J. Biol. Chem., vol. 37, p. 557.

REED, L. L., F. YAMAGUCHI, W. E. ANDERSON, AND L. B. MENDEL 1930 Factors influencing the distribution and character of adipose tissue in the rat. J. Biol. Chem., vol. 87, p. 147.

SCHNEIDER, E. C., R. W. CLARKE, AND G. C. RING 1927 The influence of physical training on the basal respiratory exchange, pulse rate and arterial blood pressure. Am. J. Physiol., vol. 81, p. 255.

SCHNEIDER, E. C., AND A. O. FOSTER 1931 The influence of physical training on the basal metabolic rate of man. Am. J. Physiol., vol. 98, p. 595.

SMITH, H. M. 1922 Gaseous exchange and physiological requirements for level and grade walking. Carnegie Inst. Wash. Pub. no. 309.

THE EFFECTS OF SOME EXTERNAL FACTORS UPON THE METABOLISM OF THE RAT [1]

KATHRYN HORST, LAFAYETTE B. MENDEL AND FRANCIS G. BENEDICT

Nutrition Laboratory, Carnegie Institution of Washington, Boston, Laboratory of Connecticut Agricultural Experiment Station, and Department of Physiological Chemistry, Yale University, New Haven

ONE FIGURE

(Received for publication May 17, 1933)

In our observations on rats covering more than 3 years of research, data have been obtained concerning the zone of thermic neutrality for basal metabolism measurements, the reaction of the adult and the young, growing rat to low environmental temperatures as expressed by the percentage increase in metabolism per degree lowering of temperature, the influence of time of day, and the effects of light and dark upon the basal metabolism.

Male and female albino rats from the Osborne and Mendel colony were used in all experiments except the second series of light and dark studies, when female rats from the colony of the Bussey Institution of Harvard University were kindly furnished by Prof. W. E. Castle. Oxygen consumption was usually measured with a multiple-chamber respiration apparatus (Benedict, '30). On one series (experiments on the influence of light and dark), carbon dioxide elimination was determined with a simple form of respiration chamber which has already been described by Benedict and Petřík ('30). The rats were usually maintained at an environmental temperature of 23° to 25°C. When the metabolism was to be measured

[1] The data in this paper are taken from a dissertation presented by Kathryn Horst in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Yale University, 1931.

at thermic neutrality, they lived in a glass cage at 26° or 28°C. for 24 hours before the metabolism experiments. In studies on the effect of a lower temperature, the rats lived at that temperature during the 24-hour preparatory period, i.e., rats 23 to 34 lived at 16° and rats 35 to 38 at 20°C. Water was always available during the fasting periods. In experiments not concerned with diurnal changes, metabolism was measured between 10 A.M. and 4 P.M. The rats were studied individually in the respiration chamber, except in the second light and dark series, when two rats huddling were measured. The heat production was calculated as explained in previous papers (Horst, Mendel and Benedict, '30, '32, '34).

DIURNAL VARIATIONS IN METABOLISM

Rubner has emphasized that metabolism measurements should cover 24 hours and that unless 24-hour measurements are made, the data are not suitable for the study of his surface-area law. Slonaker ('24) and Richter ('27), however, have shown that the activity of male and female rats differs from day to day. Hence the comparison of two rats on the basis of 24-hour measurements will obviously always show a greater degree of variability in metabolism than the comparison of two rats studied in shorter periods under complete repose. Stier ('30) in 12-hour experiments on mice (40 days old) found variations in the type of activity in different experiments. Rubner worked with fasting animals in his 24-hour measurements of basal metabolism. Our observations on the diurnal variation in the rat's metabolism, however, were incidental to a study of the effect of food upon metabolism. Four rats (table 1) were measured in consecutive periods from 2 A.M. to 10 P.M. Prior to these observations food had been withheld for 9 hours, was subsequently accessible for 4½ hours, and then was not available for 2 hours. The metabolism of each rat was high at about 2 A.M., or 2 hours after food, but tended to decrease thereafter, and minimum values were observed between 10 A.M. and 4 P.M. Unquestionably, the high metabolism in the first periods of

TABLE 1

The metabolism of rats at 28°C., in the first 20 hours after withdrawal of food

RAT NO., SEX, AGE AND WEIGHT[1]	PERIOD OF OBSERVATION		HOURS AFTER FOOD[2]	ACTIVITY	O_2 CONSUMED	
	Duration	Minutes			Total per period	Per 200 gm.[3] per minute
				Per cent	*Cc.*	*Cc.*
No. 1, ♀	2.17 A.M.– 3.43 A.M.	86	2	30	322	3.74
165 days	3.43 A.M.– 5.00 A.M.	77	3½	40	331	4.26
215 gm.	5.00 A.M.– 6.11 A.M.	71	5	45	328	4.57
202 gm.	6.11 A.M.– 7.41 A.M.	90	6	13	335	3.69
	7.41 A.M.– 9.17 A.M.	96	7½	17	332	3.42
	9.17 A.M.–10.58 A.M.	101	9	10	331	3.24
	10.58 A.M.–12.36 P.M.	98	10½	9	330	3.34
	12.36 P.M.– 2.25 P.M.	109	12½	14	336	3.05
	2.25 P.M.– 4.04 P.M.	99	14	15	328	3.28
	4.04 P.M.– 5.38 P.M.	94	16	18	321	3.38
	5.38 P.M.– 6.56 P.M.	78	17½	35	321	4.07
	6.56 P.M.– 8.18 P.M.	82	18½	23	329	3.97
	8.18 P.M.– 9.42 P.M.	84	20	13	328	3.87
Total		19 hr. 25 min.			4272	
No. 2, ♀	2.15 A.M.– 3.28 A.M.	73	2	34	327	3.88
157 days	3.28 A.M.– 4.40 A.M.	72	3	36	325	3.91
247 gm.	4.40 A.M.– 5.59 A.M.	79	4½	27	328	3.59
231 gm.	5.59 A.M.– 7.28 A.M.	89	5½	7	354	3.44
	7.28 A.M.– 8.48 A.M.	80	7	11	310	3.36
	8.48 A.M.–10.12 A.M.	84	8½	5	330	3.41
	10.12 A.M.–11.50 A.M.	98	10	6	331	2.92
	11.50 A.M.– 1.34 P.M.	104	11½	11	334	2.78
	1.34 P.M.– 3.19 P.M.	105	13½	11	329	2.72
	3.19 P.M.– 4.43 P.M.	84	15	33	325	3.35
	4.43 P.M.– 6.01 P.M.	78	16½	49	324	3.60
	6.01 P.M.– 7.10 P.M.	69	18	30	321	4.03
	7.10 P.M.– 8.15 P.M.	65	19	43	331	4.41
	8.15 P.M.– 9.24 P.M.	69	20	28	335	4.20
Total		19 hr. 9 min.			4604	
No. 3, ♂	2.12 A.M.– 3.36 A.M.	84	2	35	318	5.05
79 days	3.36 A.M.– 5.18 A.M.	102	3½	17	324	4.24
157 gm.	5.18 A.M.– 6.53 A.M.	95	5	17	332	4.66
150 gm.	6.53 A.M.– 8.46 A.M.	113	6½	8	339	4.00
	8.46 A.M.–10.38 A.M.	112	8½	11	330	3.93
	10.38 A.M.–12.27 P.M.	109	10½	16	330	4.04
	12.27 P.M.– 2.18 P.M.	111	12	18	337	4.05
	2.18 P.M.– 4.04 P.M.	106	14	16	317	3.99
	4.04 P.M.– 5.45 P.M.	101	16	23	322	4.25
	5.45 P.M.– 7.18 P.M.	93	17½	20	325	4.66
	7.18 P.M.– 8.55 P.M.	97	19	30	338	4.65
Total		18 hr. 43 min.			3612	
No. 4, ♂	3.53 A.M.– 5.40 A.M.	107	3½	12	342	4.50
76 days	5.40 A.M.– 7.29 A.M.	109	5½	7	322	4.16
152 gm.	7.29 A.M.– 9.25 A.M.	116	7	11	333	4.04
142 gm.	9.25 A.M.–11.38 A.M.	133	9	6	331	3.50
	11.38 A.M.– 1.37 P.M.	119	11½	25	333	3.94
	7.02 P.M.– 8.53 P.M.	111	19	22	332	4.21

[1] First weight in each case is weight at start and second is that at end of experiment.

[2] Estimated from time of withdrawal of food to beginning of period of observation; result is expressed to nearest half hour.

[3] Based on weight of rat at close of experiment.

measurement was in part the result of the after-effect of food but in greater part the result of excessive activity.

To ascertain whether the basal level had been reached in these experiments, the heat production per square meter of body surface per 24 hours has been calculated for each rat from the measured oxygen consumption during three to five consecutive low periods in the middle of the day. These heat values (italicized and entitled 'special measurement') and the values obtained in previous and subsequent determinations 24 hours after food under basal conditions are given in table 2. Comparison of these results shows that, whereas the heat production of rats 2 and 4 in the 'special measure-

TABLE 2

A comparison of the metabolism of rats (at 30°C.) 7 to 10 hours after food with that 24 hours after food

RAT NO., SEX, AND METABOLISM MEASUREMENT	AGE	WEIGHT	PERIOD OF OBSERVATION	HOURS AFTER FOOD	HEAT PRODUCTION PER 24 HOURS	
					Per 200 gm.	Per square meter
	Days	*Gm.*			*Cal.*	*Cal.*
No. 1, ♀						
Previous basal	134	196	10.12 A.M.–4.29 P.M.	24½	18.5	590
Previous basal	148	202	9.54 A.M.–3.31 P.M.	24	19.5	629
Special measurement	165	202	9.17 A.M.–4.04 P.M.	*9*	*21.9*	*705*
Subsequent basal	172	202	9.50 A.M.–3.46 P.M.	23	19.1	617
No. 2, ♀						
Previous basal	126	213	10.03 A.M.–3.02 P.M.	24½	21.3	701[1]
Previous basal	154	234	9.47 A.M.–2.24 P.M.	23	21.0	711
Special measurement	157	231	10.12 A.M.–3.19 P.M.	*10*	*19.0*	*640*
Subsequent basal	164	236	9.53 A.M.–3.19 P.M.	23	17.9	607
No. 3, ♂						
Previous basal	72	137	10.47 A.M.–4.48 P.M.	18	27.2	769
Special measurement	79	150	6.53 A.M.–4.04 P.M.	*6½*	*27.1*	*791*
Subsequent basal	86	168	9.56 A.M.–5.04 P.M.	17	25.6	776
No. 4, ♂						
Previous basal	62	108	10.07 A.M.–3.44 P.M.	18½	24.3	636[1]
Special measurement	76	142	9.25 A.M.–1.37 P.M.	*9*	*25.2*	*722*[1]
Subsequent basal	81	158	9.59 A.M.–5.56 P.M.	17	24.3	722

[1] Denotes metabolism with activity in excess of 15 per cent.

ment' reached a basal level in 9 to 10 hours after food, the metabolism of rats 1 and 3 in the 'special measurement' remained somewhat above basal values. Although the exact time when food was eaten is not known, it is probable that at the time of the special measurements our rats had been fasting from 3 to 4 hours longer than is indicated in tables 1 and 2. Furthermore, the rats may have consumed considerable food in the first hour when food was available and none in the last 3½ hours.[2] In that event rats 2 and 4 would have been without food from 12 to 14 hours when the metabolism (special measurement) reached a basal level. Wesson ('31) and v. Árvay and Verzár ('28) found that the heat production of the rat was at a minimum in 14 to 16 hours after food. Benedict and MacLeod ('29) noted that the metabolism of the rat decreased during the first 17 to 24 hours after food and remained essentially constant thereafter up to the sixty-fourth hour. Moreover, Horst, Mendel, and Benedict ('30) observed respiratory quotients not far from 0.72 in 16 to 23 hours after food. Hence it appears that the ingestion of food does not exert any appreciable influence on the rat's metabolism after 14 to 17 hours.

The total heat production of three of our rats for the 24-hour cycle has been estimated from the data in table 1 by projecting the oxygen value (total per period) observed at about 2 A.M. back to midnight and at 9 or 10 P.M. forward to midnight and calculating the heat production from the total volume of oxygen consumed in 24 hours. On this basis the metabolism per 200 gm. of body weight per 24 hours was 24.8, 24.3, and 29.9 calories for rats 1, 2, and 3, respectively. The heat production of these same rats in short periods at midday under quiet basal conditions averaged only 19.0, 20.1, and 26.4 calories, respectively (table 2). On the basis of body surface the heat values are 799, 819, and 872 calories

[2] During the 4½ hours when food was accessible, rat 1 consumed 3 gm. of a special dog biscuit and 20 gm. fresh lettuce; rat 2, 7 gm. of the biscuit and 20 gm. fresh lettuce; rat 3, 4 gm. of a synthetic diet, and rat 4, 7 gm. of the same diet. Rats 3 and 4 each ingested 200 mg. dry brewers' yeast 2 hours before oxygen consumption was measured.

(24-hour measurements) and 612, 673, and 773 calories (basal measurements several hours in duration). Thus the metabolism based on 24-hour measurements was from 13 to 31 per cent higher than that calculated from 4- to 6-hour measurements made during midday.

The metabolism of rat 2 (table 1) was 50 per cent higher, that of rat 1 was 24 per cent higher, and that of rat 3 was 13 per cent higher after 4 P.M. (17 to 20 hours after food) than it was during midday (9 to 12 hours after food). This higher metabolism after 4 P.M. cannot be attributed to the after-effect of food. It may be coincident with an increased activity associated with the normal feeding habits of the animals. Food was accessible to these rats (except when fasting for an experiment) at all hours, but fresh food was always placed in the cages between 3 and 5 P.M. Szymanski ('18) found that his rats were usually active less than 25 per cent of the time between 6 A.M. and 4 P.M., whereas from 4 P.M. to 6 A.M. they were active more than 25 per cent of the time. The activity of our rats, especially rats 1 and 2, corresponds to the diurnal variations observed by Szymanski.

Our experimental evidence is so obscured by the uncontrollable muscular activity of the rats that the question as to whether there is a diurnal variation in metabolism irrespective of the activity accompanying the night period remains unsolved. Undoubtedly, throughout the night our rats were subjected to a less degree than during the day to external stimuli of noises around the laboratory. But they were most active at night. It is striking that in the daytime with the various noises in the street and in the building the rats retained as low a metabolism as was found for the most part in these experiments. Perhaps the most important finding in this study is that it is practically out of the question to determine the basal metabolism of rats for comparative purposes before 10 A.M. and after 4 P.M.

METABOLISM IN ABSOLUTE DARKNESS DURING MIDDAY

Earlier observations at Columbia University (Benedict and MacLeod, '29), made during the day, indicated that rats were quieter in a respiration chamber of glass than in complete darkness. Szymanski ('18) found that rats were active for a longer time during the 24-hour cycle when in complete darkness than when exposed to the diurnal variations of light and dark. Moleschott and Fubini (1881) reported carbon-dioxide excretion higher in the light than in the dark. On the other hand, Bergfeld ('31) observed that the metabolism of rats living in darkness was essentially the same as that of animals exposed to various conditions of light. Benedict and Riddle ('29) found indications that light increased the metabolism of ring doves, although their kymograph records gave evidence that the birds were more active in the light than in the dark. Kestner, Johnson and Laubmann ('31) have shown that human metabolism is elevated by exposure to sunlight. It is evident that in a study of the effect of darkness or light upon the metabolism of the rat, muscular activity must be ruled out. Our rats were studied in complete darkness and in light between 10 A.M. and 4 P.M., as we realized they would be reasonably quiet between these hours. In one series the oxygen consumption of four adult male rats was measured on 3 separate days, some days in the light and other days in the dark. In another series the carbon-dioxide elimination of young female rats was measured in consecutive half-hour periods with alternate exposure, 1 hour to the light and 1 hour to the dark.

The heat production of the male rats (table 3) was uniformly high on the first day, regardless of whether light or darkness prevailed. On the last 2 days, rat 5 had a heat production 10 per cent higher, rat 6, 4 per cent higher, and rat 8, 4 per cent lower in darkness than in light. Rat 7 showed no change. The kymograph records indicated that rat 6 was quieter in the light than in the dark, but on the whole it was not very restless in the dark. Rats 5, 7, and 8 were quiet both in the light and in darkness. The day to day

variations in heat production of these rats measured one day in the light and another day in the dark lie well within the limits of variation observed in measurements under standard conditions, and suggest that the basal metabolism of the rat is unaltered by exposure to complete darkness for 4 to 6 hours during midday.

TABLE 3

The influence of light and dark on the metabolism of male rats[1]

RAT NO. AND AGE	WEIGHT	CONDITION OF CHAMBER	ACTIVITY	HEAT PRODUCED PER 24 HOURS	
				Per 200 gm.	Per square meter
	Gm.		*Per cent*	*Cal.*	*Cal.*
No. 5					
262 days	503	Light	8	18.5	807
274 days	507	Dark	5	17.7	775
281 days	510	Light	4	16.0	701
No. 6					
262 days	439	Dark	10	16.6	690
274 days	434	Light	7	15.1	626
281 days	433	Dark	16	15.6	651
No. 7					
292 days	474	Light	5	16.6	714
299 days	473	Dark	6	15.5	661
306 days	473	Light	4	15.5	665
No. 8					
287 days	446	Dark	7	18.2[2]	763[2]
294 days	440	Light	7	14.8	620
301 days	431	Dark	7	14.4	595

[1] Measurements were made at 28°C., between 10.30 A.M. and 3.00 P.M., and 24 hours after food.

[2] During this experiment the rat broke a hinge on its cage door.

In the second series (table 4) female rats were studied that weighed one-third as much as the males in the first series. The average heat production of rats 9 and 10 in experiment I was 30.4 calories per 200 gm. per 24 hours during the first hour with exposure to light. During the next hour, in the dark, it decreased to 26.8 calories and remained essentially at that level during the third hour with exposure to light.

In the second experiment, the average heat production of rats 11 and 12 was approximately 32 calories both during ½ hour in the light and 1 hour in the dark. Throughout the next 3 hours, when the rats were in the light the first hour, in the dark the second hour, and in the light again the third hour, the metabolism was considerably lower, about 25

TABLE 4

The influence of light and dark on the metabolism of female rats[1] *(18 to 24 hours after food)*

EXPERIMENT NO., RATS, AND WEIGHTS	TIME OF DAY (30-MINUTE PERIODS)	CONDITION OF CHAMBER	ACTIVITY[2]	CO_2 PRODUCED PER 30 MINUTES	HEAT PRODUCED PER 24 HOURS	
					Per 200 grams	Per square meter
			Per cent	*Gm.*	*Cal.*	*Cal.*
Experiment I						
Rat 9, 139 gm.	10.24 A.M.–10.54 A.M.	Light	6	0.2863	30.4	882
Rat 10, 154 gm.	10.54 A.M.–11.24 A.M.	Light	2	0.2723		
	11.37 A.M.–12.08 P.M.	Dark	....	0.2445	26.8	777
	12.08 P.M.–12.38 P.M.	Dark	....	0.2466		
	12.40 P.M.– 1.10 P.M.	Light	1	0.2360	26.0	754
	1.10 P.M.– 1.40 P.M.	Light	2	0.2415		
Experiment II						
Rat 11, 152 gm.	9.53 A.M.–10.23 A.M.	Light	13	0.3090	32.1	944
Rat 12, 155 gm.						
	10.27 A.M.–10.57 A.M.	Dark	....	0.3119	32.4	953
	10.57 A.M.–11.27 A.M.	Dark	....	0.3109		
	11.30 A.M.–12.00 M.	Light	0	0.2408	24.4	718
	12.01 P.M.–12.31 P.M.	Light	1	0.2283		
	12.34 P.M.– 1.04 P.M.	Dark	....	0.2525	25.4	747
	1.04 P.M.– 1.34 P.M.	Dark	....	0.2362		
	1.47 P.M.– 2.18 P.M.	Light	0.4	0.2428	25.0	735
	2.18 P.M.– 2.48 P.M.	Light	1	0.2380		
	2.51 P.M.– 3.21 P.M.	Dark	....	0.2771	27.4	806
	3.21 P.M.– 3.51 P.M.	Dark	....	0.2506		

[1] Two rats in same chamber, huddling, were measured at 30° to 31°C. in experiment I and at 29° to 30°C. in experiment II.

[2] Ascertained by ocular observation during the light periods only.

calories. During the last hour, in the dark, the metabolism was higher again. The heat production varied more with the time of day than with conditions of light and dark. Moreover the low metabolism observed during midday persisted throughout a short exposure (1 hour) to absolute darkness. Our findings for the rat are in accordance with the observations of Johnson ('26) who showed that the 24-hour rhythm of activity of wild deer-mice persisted in the absence of the daily change of light and dark.

INFLUENCE OF SEX AND AGE

The basal heat production at 30° of our adult female rats[3] (nos. 1 and 2, 172 and 164 days old, table 2) was not far from 600 calories per square meter per 24 hours. Horst, Mendel, and Benedict ('30) have reported values of 600 to 700 calories for female rats about 200 days of age, measured at 28° and 24 hours after food. The heat production of male rats of the same age (unpublished data) has been found to be about 725 calories. The metabolism of our smaller females[4] (evidently younger, but the ages are not known) averaged 765 calories[5] (rats 9 and 10, table 4) and 733 calories[6] (rats 11 and 12). According to Donaldson's ('24) standards these females, which weighed from 139 to 155 gm., were probably not far from 90 days old. The heat production of our male rats from 92 to 101 days old (table 7) averaged 786 calories per square meter. The average heat production of our four adult males at 280 days of age and weighing from 431 to 510 gm. (table 3) was 689 calories—a value similar to the 664 calories reported previously for the male rat weighing from 400 to 560 gm. (Benedict, Horst and Mendel, '32). Thus the metabolism of the young females was 5 per cent lower and that of the adult females, 17 per cent lower than that of the males, and with both sexes the metabolism decreased with

[3] From the Osborne and Mendel colony.

[4] From the colony of Prof. W. E. Castle at the Bussey Institution of Harvard University.

[5] On the basis of carbon dioxide produced from 11.37 A.M. to 1.40 P.M.

[6] On the basis of carbon dioxide produced from 11.30 A.M. to 2.48 P.M.

age. This same picture of decreasing metabolism with advancing age is shown in table 7.

THERMIC NEUTRALITY

In recent years many investigators have followed the excellent example of Hári ('24), Aszódi ('24) and others of the Tangl school at Budapest, who study the metabolism of the rat at an environmental temperature of 28°C. Examination of the literature, however, shows that the temperatures preferred in the different laboratories range from 26° (Asher and Honda, '27) to 32° and 33°C. (Terroine and Trautmann, '27). Fraser and Wiesner ('29–'30) have actually reported measurements on the rat at 37°C. Some of the variations in the conditions of thermic neutrality in the different laboratories can probably be explained by the different procedures employed in ascertaining the temperature of the environment. A thermometer near the rat will unquestionably be affected by the animal's temperature. It would be ideal to use a resistance thermometer wound entirely around the wire cage in which the animal is placed. The difficulty of incorporating this electrical equipment was so great that it was not used in our measurements. We experimented with a number of small thermometers attached to different parts of the animal's cage, one near the rat, one above it, one on the floor of the chamber (with the heating element functioning beneath the chamber), and one at the exit end of the chamber for the ventilating current and at least 2 cm. above the rat cage. The latter position was accepted as giving the most uniform temperature. The temperatures recorded by these four thermometers showed a difference of approximately 1°C. In all probability the records that we used of the temperature determined at least 2 cm. above the rat cage were within 1°C. of the average chamber temperature.

Goto ('23), Terroine and Trautmann ('27) and Houssay and Artundo ('29) have shown that above 28° or 30°C. the heat production of rats increases. Four of our male rats from 50 to 100 days of age were measured at 28° or 30° on

one day and at 32° on another day.[7] Three males from 200 to 300 days old were likewise measured at 30° on one day and at 32°C. several weeks later. Some of the determinations were made with calcium chloride in the respiration chamber, to reduce the moisture content of the air. In another series

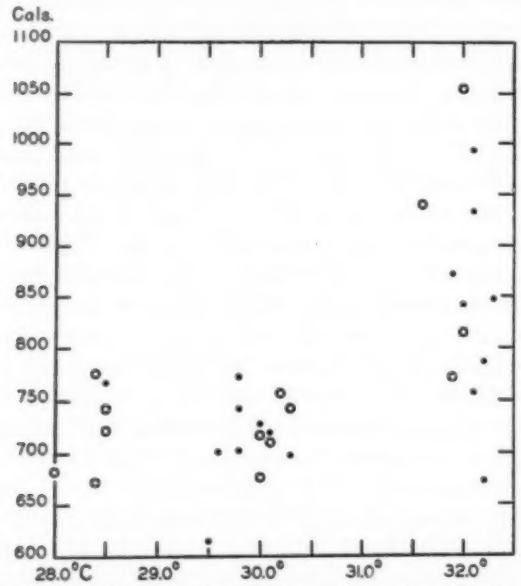


Fig. 1 Heat production of male rats per square meter of body surface per 24 hours referred to environmental temperature. The open circles represent determinations made without calcium chloride and the solid circles those made with calcium chloride in the respiration chamber.

the metabolism of three adult males about 240 days of age was measured at 30°, on some days with and on other days without calcium chloride in the chamber. The results are plotted in figure 1, the abscissae representing the environmental temperature and the ordinates the calories per square meter of body surface per 24 hours. The open circles represent the determinations made without calcium chloride and

[7] The number of days between experiments varied from 4 to 21.

the solid dots those with calcium chloride. With one exception the metabolism of these rats was higher at 32° than at 28° or 30°C., the total increase averaging 30 per cent for three adult rats and two young rats or 15 per cent per degree (Centigrade) that the environmental temperature increased above 30°C. Three young rats measured at 28° and 32° had a total increase in metabolism of 10.5 per cent, or 2.6 per cent per degree increase in environmental temperature above 28°C. According to the kymograph records the young rats especially were more restless at 32° than at 28° or 30°C. Undoubtedly, the higher metabolism can be attributed to a great extent to the increase in activity. Thus it appears that with rats, measurements at 32° give too high results and that the most ideal temperature for measuring the basal metabolism of rats is at 28° to 30°C. Within these limits there is no appreciable variation in metabolism. With our young female rats (unpublished data) the metabolism was also essentially the same at 28° and at 30°C.

Humidity. It is conceivable that at 32° the moisture content of the air in the respiration chamber might have become excessive and that the normal path for heat loss by vaporization of water was disturbed. Greene and Luce ('31) did not observe a change in the percentage of heat lost by vaporization of water in the rat with variations in environmental temperature, but the range under observation, 25° to 31°C. was lower than the temperatures studied by us. Our observations were usually made with calcium chloride in the respiration chamber. When calcium chloride was not used, especially with a large rat, moisture frequently condensed on the inside of the glass chamber, even though the rate of ventilation was 2 liters per minute. A small tray filled with calcium chloride was placed upon the top of the cage above the rat, but at the opposite end from the thermometer so that the heat of absorption of water vapor could not influence the thermometer. As seen from figure 1, the metabolism at 32° was consistently high and that at 30° was always lower, regardless of the variations in the humidity as determined by the presence or

absence of calcium chloride. Our results confirm the earlier report by MacLeod ('07), who observed that at 30° the carbon-dioxide excretion in rats was the same with exposure to dry or moist air and at 33° it was higher than at 30°, both in dry and in moist air. Our experiments were far from a repetition of or an attempt to confirm the extensive studies of Sundstroem ('30), who compared the basal metabolism of rats living at 32° in an atmosphere having a relative humidity of 70 to 80 per cent with the metabolism of rats living at 32° in a dry atmosphere. Since Sundstroem used wet kata cooling power as an index of the climatic environment of his rats, it is impossible to compare his results with ours, as our method of temperature control was different.

The difficulty of obtaining measurements on the rat in complete repose, regardless of variations in temperature and humidity of the environment, complicates the study of the effects of environmental factors per se. In some of our experiments we attempted to obtain greater repose by caging the rats in perforated celluloid cylinders. When the rat fitted tightly into the cylinder, the metabolism was higher than normal. If the cylinder was slightly larger than the rat, the metabolism was essentially the same (unpublished data) as when the rat was measured in the regular metal cage. Frequently, the rat struggled in an attempt to change its position in the cylinder, at times resting on its back, at times on its side, but rarely ever in an upright position. Herzog ('30) with birds and Sundstroem ('30) with rats likewise found increased irritability and tonus when their animals were measured in cages small enough to prevent normal movements.

Rectal temperatures. The rectal temperatures of the adult rats determined immediately after the metabolism measurements were from 0.5° to 1.2°C. higher at an environmental temperature of 32° than at 30°C. The higher cell temperature of the rat would in itself tend toward an increased metabolism, in conformity with our findings with the respiration chamber.

THE INFLUENCE OF ENVIRONMENTAL TEMPERATURE UPON THE METABOLISM OF ADULT RATS

The basal metabolism of twelve male rats (127 to 150 days old) was established at 30°, when they had been living for 40 days at 21° to 27°C. The animals were then exposed to 16° for 10 days, when the metabolism was measured again at temperatures between 15.1° and 19.8°C. This procedure is in striking contrast to the quick transition from one temperature to another or the short exposure to the special environmental temperature, methods used, among others, by Benedict and Petřik ('30), Houssay and Artundo ('29) and Benedict and MacLeod ('29). Our rats had been used previously for a study of the effect of the protein level upon the basal metabolism. Our results introduce changes in metabolism, therefore, incident to the protein factor. The rats fasted, however, 24 hours before the metabolism was measured, so that the effect of protein was not an immediate after-effect corresponding to the specific dynamic action but a more persistent effect caused by changes in body composition resulting from extreme variations in the protein intake. The time between the measurements at the high and the low temperatures was so short that the age element did not enter to the extent that it did in the case of the growing rats discussed on pages 295 to 300. In estimating the increase in metabolism per degree fall in environmental temperature, we have used the exact chamber temperatures in each case (table 5) instead of the average values 30° and 17°C.

The data summarized in the first half of table 5 contribute information regarding the basal metabolism of the adult male rat, for the conditions of measurement, particularly as regards environmental temperature, were those stipulated for basal measurements. At 30°C. the heat production per square meter of body surface ranged from 603 to 742 calories. The latter value admittedly reflects the influence of activity greater than that noted in most of the other cases. The average metabolism of the entire series at 30° was 682 calories. Some values reported recently by other investigators are 718

TABLE 5

Metabolism of adult male rats[1] at 30° and 17°C.

RAT NO.	PROTEIN IN PREVIOUS DIET	30°C.				17°C.				INCREASE IN METABOLISM PER DEGREE FALL IN ENVIRONMENTAL TEMPERATURE	
		Weight	Rectal temperature	Chamber temperature	Calories per square meter per 24 hours	Weight	Rectal temperature	Chamber temperature	Calories per square meter per 24 hours	Calories	Per cent
		Gm.	*°C.*	*°C.*		*Gm.*	*°C.*	*°C.*			
28	Medium	230	37.4	29.7	672[2]	237	37.8	19.8	1243	58	8.6
23	High	274	37.3	28.9	643	297	37.9	19.4	1236	62	9.6
31	Low	250	37.1	29.6	631	240	37.1	19.0	1184	52	8.2
24	High	251	36.9	29.4	742[2]	266	37.0	17.6	1244	43	5.8
29	Medium	317	36.9	29.7	704[2]	300	38.2	17.2	1420[2]	57	8.1
26	High	317	36.9	29.1	686	295	37.6	15.9	1349	50	7.3
33	Low	256	37.6	29.8	603	238	38.3	15.9	1295	50	8.3
32	Low	197	38.0	30.0	651	213	38.8	15.6	1359[2]	49	7.5
30	Medium	282	37.3	29.7	707	266	37.3	15.4	1238	37	5.2
34	Low	254	37.0	29.5	693	249	37.0	15.4	1381	49	7.1
27	Medium	238	37.4	30.0	724	250	38.0	15.3	1350	43	5.9
25	High	284	37.2	30.0	725[2]	279	36.9	15.1	1328	40	5.5
Aver.		263	37.3	29.6	682	261	37.7	16.8	1302	49	7.3

[1] Rats measured when they had been 24 hours without food, at 28° or 16°C. (p. 278).

[2] Rat active more than 15 per cent of the time of measurement.

calories (Benedict and MacLeod, '29) and 805 calories (Verzár, '29).

The data in the second half of table 5 indicate the metabolism of the same rats at a relatively low environmental temperature of 17°C. and thus add to our general knowledge of the metabolism of animals of various sizes and zoölogical groups at 16° or 17°, the temperature at which Rubner made his measurements on different animals for formulating the surface-area law. Secondly, since the metabolism of these rats was likewise well established at 30°, the measurements at 17° give direct information with regard to the temperature coefficient or the increase in metabolism per degree fall in environmental temperature. The metabolism of our twelve rats was, on the average, nearly twice as great at 17° as at 30°C., 1302 calories as compared with 682 calories per square meter. It may be questioned whether values obtained at environmental temperatures ranging from 15.1° to 19.8° should be averaged. The heat values at the temperatures around 15° are higher, however, than those at the temperatures of 19°. Hence, the average value of 1302 calories probably represents what would have been the average metabolism of these rats had they been measured at 16.8°C. In only one instance did the activity exceed 20 per cent of the total time of measurement. Evidently there was no pronounced variation in activity ascribable to differences in environmental temperature.

The interval between the two temperatures at which the rats were studied varied from 9.5° with rat 23 to nearly 15° with rat 25. On the average the metabolism increased 7.3 per cent per degree decrease in environmental temperature below 30°C. This percentage increase corresponds to that noted by Houssay and Artundo ('29), who reported for 180- and 219-gm. rats a heat production of 86 calories per square meter per hour at 15° and 42 calories at 30°C., or an increase of 7 per cent per degree decrease from 30° to 15°C. Our rats were acclimatized to 16° and those of Houssay and Artundo were suddenly exposed to the low environmental temperature.

If the data reported by Benedict and MacLeod ('29) for rats at 10.9° to 31.7° are calculated on the basis of the measured minimum metabolism, the increase in metabolism is nearly 6 per cent per degree decrease in temperature below 28°C. Our rats acclimatized to 16° showed, on the whole, a greater increase in metabolism with exposure to low environmental temperature than that observed by other investigators who studied the immediate reaction of the rat to cold. The metabolic reaction of the rat to low temperatures resembles that of the dog and the guinea pig—species in which Rubner long ago found variations in metabolism with changes in environmental temperature.

The average value of 1302 calories per square meter of body surface noted with our twelve rats at 17° (based upon careful measurements with the most perfected technic) is presumably comparable to values found by Rubner and other research workers at 16°, the temperature at which the surface-area law was supposed especially to apply. Since our measurements were made during the period of minimum activity of the rat and in relatively short periods, it can be readily argued that 1302 calories represents the minimum value for the 24-hour metabolism at 17° and that if the rats had been measured during ordinary cage activity and over a 24-hour period, this value would have been very appreciably increased and would bear no relationship to the supposedly constant value of 1000 calories per square meter of body surface suggested for all warm-blooded animals at 16°C.

Rectal temperatures. The rectal temperatures of the twelve rats (table 5) at the end of 30 hours at 30° averaged 37.3° and ranged from 36.9° to 38.0°C. At 17° they averaged 37.7° and ranged from 36.9° to 38.8°C. Thus the rectal temperatures were lowest in the warm environment, but the differences were slight. The decrease in rectal temperature coincident with sudden exposure to 32° and 33° noted by Giaja, Chahovitch and Males ('28) represents the immediate reaction of the rat to a change in environmental temperature, whereas our experiments were concerned with the rectal tem-

peratures of rats acclimatized to two different environmental temperatures.

THE INFLUENCE OF ENVIRONMENTAL TEMPERATURE UPON THE METABOLISM OF THE GROWING RAT

In a consideration of the effect of environmental temperature upon the metabolism of the growing rat at least two other factors must be recognized, constantly changing age and variation in size. It is usually assumed that differences in heat production caused by variations in size are equalized by expressing the results on the basis of body surface rather than body weight. However, variations in metabolism may occur with changes in age even at thermic neutrality. Moreover, the young rat may not react to low environmental temperature as does the adult rat. Hill and Hill ('13) found that at 14° the heat production per unit of surface area was 40 per cent higher in rats weighing 50 to 90 gm. than in rats weighing 90 to 130 gm. On the other hand, Mitchell and Carman ('26) did not find any essential difference at 28° to 31°C. in male rats from 91 to 188 days old. Houssay and Artundo ('29) observed that at 20° and at 0°C. small rats (100 to 179 gm.) had a somewhat higher metabolism per unit of surface area than large rats (180 to 320 gm.), but that at 30° the metabolism of rats weighing 100 to 320 gm. was essentially the same. Benedict and MacLeod ('29) noted that at 26° and 29°C. the metabolism of female rats from 60 days to over 2 years old was higher, the older the rat. The results obtained by Kayser ('30) in his excellent studies on rats (results recomputed by us from calories per kilogram to calories per square meter) show that at 29° three young rats and one adult had essentially the same metabolism, that at 21° a young rat had a lower heat production than an adult rat, and that the metabolism of his young rats was increased 7 per cent and that of his adult rats 11 per cent per degree decrease in environmental temperature from 29° to 20°C. The differences observed by the various investigators in the reaction of growing rats to low environmental temperatures

were probably caused by the different lengths of time that the rats lived at the particular temperatures. Our rats lived at a low temperature for several weeks before the metabolism was measured.

Four rats that had lived 7 days at 25° and were 42 to 44 days old were measured at 30°C. They then lived for 56 days at 20°, and their metabolism was measured at this temperature on the twenty-third and the fifty-sixth days when they were 66 and 99 days old, respectively. After 56 days at 20° they lived for 33 days at 25°, when they were again measured (average age, 133 days) at 30°C.

The first series of experiments made at 30° (table 6) show that with three of the 43-day-old male rats (the fourth rat was too active for comparative purposes) the average heat production per square meter of body surface was 913 calories. This is a relatively high metabolism in comparison with that of 850 calories observed on male rats 39 to 45 (table 7) of the same age and sex. Since the relatively high metabolism of 913 calories was noted with rats 35, 36, and 37 before they were exposed to 20°, the high heat production cannot be attributed to an after-effect of the stay at 20°C. The rats in both groups lived at 25°, were measured at 30°, and received the same diet, but rats 39 to 45 weighed considerably more (average weight, 90 gm.) at 45 days of age than rats 35 to 38 at the same age. In this and other papers the relatively great differences in weights of rats for the same age ascribable either to differences in feeding or to racial characteristics have presented difficulties in the method of calculating the basal metabolism for purposes of comparison, and challenge the legendary sufficiency of the calculations upon the basis of the surface area.

At 133 days of age (table 6) the rats measured at 30° had an average heat production of 725 calories and an average weight of 256 gm. Another group of rats, which may be considered as a control group (table 7), 135 days old and weighing 239 gm., had an average heat production of 756 calories or essentially the same as rats 35 to 38. Indeed, both these

groups of male rats had a metabolism not appreciably higher than the average of 708 calories previously reported (Benedict, Horst and Mendel, '32) for the adult male rat weighing from 300 to 400 gm. Therefore, only with the very young rats did the striking differences in weight and in metabolism

TABLE 6

The effect of environmental temperature upon the metabolism of growing male rats (24 hours after food)

RAT NO.	AT 30°C.[1]				AT 20°C.[2]			
	Age	Weight	Rectal temperature[3]	Calories per square meter per 24 hours	Age	Weight	Rectal temperature[4]	Calories per square meter per 24 hours
	Days	*Gm.*	*°C.*		*Days*	*Gm.*	*°C.*	
35	44	79	37.9	899	67	124	38.0	1243
36	42	71	37.9	891	65	111	38.1	1224
37	42	70	38.0	948	65	119	38.3	1018
38	44	68	38.2	[5]	67	107	37.8	1132
Aver.	43	72	38.0	913	66	115	38.1	1154
35	133	281	37.9	774	100	204	37.5	1105
36	133	260	37.9	739	98	171	38.0	1039
37	131	266	38.2	694	98	190	38.1	1040
38	135	216	38.2	692	100	173	38.1	1120
Aver.	133	256	38.1	725	99	185	37.9	1076

[1] Rats lived at 25° prior to metabolism measurements.

[2] Rats lived at about 20°C. for 56 days. For 23 days before first metabolism measurement the environmental temperature ranged from 18° to 22°C. and sometimes fell as low as 15°C. For 33 days before second metabolism measurement the environmental temperature was about 20°C., except for 10 days, when it rose to 25° at times and for 1 week when it was frequently 27°C.

[3] At 43 days the rectal temperatures were determined when the rats were living at 25°C.; at 133 days after a 6-hour exposure to 30°C.

[4] Determined when the rats were living at 20°C.

[5] Rat active more than 15 per cent of the time of measurement.

for age appear. Because of these differences with the two groups of young rats, we find a difference in the age effect in the two series. Thus, according to the data in table 6, the average metabolism of the 43-day-old rats weighing 72 gm. was 26 per cent higher than that of the 133-day-old rats weighing 256 gm., whereas according to the data in table 7 the

TABLE 7

The metabolism of male rats, 42 to 138 days old, measured at 30°C., and 24 hours after food

RAT NO.	AGE	WEIGHT	HEAT PRODUCTION PER 24 HOURS	
			Per 200 gm.	Per square meter
	Days	*Gm.*	*Cal.*	*Cal.*
39	42	88	33.6	822
40	42	77	34.2	800
41	44	96	36.6	916
42	44	80	38.0	899
43	45	98	31.4	798
44	48	97	32.8	828
45	48	94	35.6	888
Average	45	90	34.6	850
46	57	114	34.0	907
40	57	102	32.5	834
47	59	118	30.1	808
41	60	138	32.0	909
48	62	100	33.4	852
49	64	125	30.5	855
43	64	134	25.8	727
50	69	125	29.2	803
Average	62	120	30.9	837
49	92	184	24.6	769
50	93	182	26.2	815
43	94	188	24.3	763
51	94	176	26.7	822
52	98	179	24.4	754
53	99	180	24.4	759
54	99	184	24.4	762
55	99	177	26.9	831
56	101	178	25.8	795
Average	97	181	25.3	786
57	131	236	21.4[1]	724[1]
43	133	236	21.0	713
58	133	237	20.2	688
51	133	248	23.6	816
40	134	224	25.5	851
56	136	247	21.7	749
47	136	252	22.3[1]	774[1]
59	137	249	21.0	725
60	138	226	22.8	763
Average	135	239	22.2	756

[1] Rat active more than 15 per cent of the time of measurement.

average metabolism of 45-day-old rats averaging 90 gm. in weight was only 12 per cent higher than that of 135-day-old rats weighing 239 gm.

The heat production at 20° can also be considered from the standpoint of variation in metabolism with advance in age and increase in weight, i.e., the metabolism at 66 days and 20° can be compared with that at 99 days and 20°C. On the basis of square meter of body surface, the metabolism of rats 35 and 36 was from 12 to 18 per cent higher at 66 than at 99 days, whereas the heat production of rats 37 and 38 was essentially the same at both ages. At thermic neutrality the average metabolism of eight rats from 57 to 69 days of age (table 7) was 6 per cent higher than the heat production of nine rats from 92 to 101 days of age. Thus at the low environmental temperature rats 35 and 36 showed a greater variation in metabolism and rats 37 and 38 less, as the age advanced from 66 to 99 days, than the average change in heat production of rats of similar ages but measured at thermic neutrality.

As the age element enters into the comparison of the values at 30° and 20° in table 6, the picture of the temperature effect is obscure, even though the results have been calculated per unit of surface area. At 66 days of age and 20° the metabolism was notably higher than at 43 days of age and 30°, in spite of the difference in age. Similarly at 99 days and 20° the metabolism was much higher than at 133 days and 30°, although in this comparison the age effect is reversed, the rats being younger when measured at 20° than at 30°C. To eliminate variations in metabolism caused by differences in age, the heat production of rats 35 to 38 at 20° may be compared with the heat production of other rats (table 7) of the same age, weight, and sex, measured at 30°C. The average values are as follows:

62 days	120 gm.	30°	837 calories per square meter
66 days	115 gm.	20°	1154 calories per square meter
97 days	181 gm.	30°	786 calories per square meter
99 days	185 gm.	20°	1076 calories per square meter

According to these averages, the metabolism of the 66-day-old rats increased 3.8 per cent and that of the 99-day-old rats 3.7 per cent per degree decrease in environmental temperature from 30° to 20°C. In our study of the effect of temperature on adult rats (table 5) the increase in metabolism per degree fall in environmental temperature was, on the average, 7.3 per cent. Hence, low environmental temperatures seemed to affect the young rats less. However, the young rats were measured at 20° whereas the adult rats were measured at lower temperatures averaging 16.8°. In any event the evidence is clear that the metabolic reaction of the young, growing rat to a low environmental temperature is not greater than that of the adult rat.

Rectal temperatures. The rectal temperatures (table 6) of the rats at 20° were much the same as those at 25° or 30°, averaging essentially 38.0°C. at both environmental temperatures. However, the range (37.5° to 38.3°) in the measurements at 20° was somewhat greater than the range (37.9° to 38.2°) in determinations at 25° or 30°C.

SUMMARY

The oxygen consumption of two adult female and two young male rats in the first 20 hours after food (2 A.M. to 10 P.M.) was high at the start and reached a minimum slightly above the basal level at midday. After 4 P.M. the metabolism increased again and in the last hours of measurement was from 13 to 50 per cent higher than during the middle of the day. The rats were restless before 6 A.M., quiet during the middle of the day, and active after 4 P.M. The 24-hour heat production of the three rats per 200 gm. of body weight, calculated from the oxygen consumption measured between 2 A.M. and 10 P.M., was from 13 to 31 per cent above the basal heat production calculated from the oxygen consumption measured under conditions of repose between 10 A.M. and 4 P.M. Hence, for comparative purposes, the basal metabolism of the rat should be measured only between 10 A.M. and 4 P.M.

The metabolism of four adult male rats and four young females did not vary with changes from daylight to complete darkness between 10 A.M. and 4 P.M.

The metabolism of adult male rats increased, on the average, 7.3 per cent per degree decrease in environmental temperature below 30°C. That of young, growing male rats increased only 3.8 per cent.

The rectal temperatures of adult males averaged 37.7° at an environmental temperature of 17° and 37.3° at 30°C. The rectal temperatures of young, growing male rats were essentially the same (38.0°C.) at 20°, 25°, and 30°C.

At thermic neutrality the metabolism of female rats was lower than that of males, both at young and adult ages, and with both sexes the metabolism decreased with age.

LITERATURE CITED

v. ÁRVAY, A., AND F. VERZÁR 1928 Die spezifisch-dynamische Wirkung bei Vitaminmangel. Biochem. Zeitschr., Bd. 192, S. 369.

ASHER, L., AND T. HONDA 1927 Beiträge zur Physiologie der Drüsen. Die Wirkung von Fleisch auf den respiratorischen Umsatz der mit Fett gefütterten Ratten. Biochem. Zeitschr., Bd. 185, S. 173.

ASZÓDI, Z. 1924 Tierische Kalorimetrie. III. Energieumsatz kleiner Tiere bei chronischer Unterernährung. Biochem. Zeitschr., Bd. 152, S. 472.

BENEDICT, F. G. 1930 A multiple-chamber respiration apparatus for rats and other small animals. J. Nutrition, vol. 3, p. 161.

BENEDICT, F. G., K. HORST, AND L. B. MENDEL 1932 The heat production of unusually large rats during prolonged fasting. J. Nutrition, vol. 5, p. 581.

BENEDICT, F. G., AND G. MACLEOD 1929 The heat production of the albino rat. I. Technique, activity control, and the influence of fasting. J. Nutrition, vol. 1, p. 343.

——— 1929 II. Influence of environmental temperature, age, and sex; comparison with the basal metabolism of man. Ibid., p. 367.

BENEDICT, F. G., AND J. M. PETŘÍK 1930 Metabolism studies on the wild rat. Am. J. Physiol., vol. 94, p. 662.

BENEDICT, F. G., AND O. RIDDLE 1929 The measurement of the basal heat production of pigeons. II. Physiological technique. J. Nutrition, vol. 1, p. 497.

BERGFELD, W. 1931 Über die Einwirkung des Ultravioletten Sonnen- und Himmelslichtes auf die Rattenschilddrüse mit Berücksichtigung des Grundumsatzes. Strahlentherapie, Bd. 39, S. 245.

DONALDSON, H. H. 1924 The rat. 2nd ed., p. 281. Philadelphia.

FRASER, A. C., AND B. P. WIESNER 1929–1930 Variations of rest metabolism of rat in relation to sex cycle. Proc. Roy. Soc., Edinburgh, vol. 50, p. 1.

GIAJA, J., X. CHAHOVITCH, AND B. MALES 1928 Sur l'abaissement de la température du rat et de la souris maintenus un certain temps à la neutralité thermique. Compt. rend. Soc. de biol., T. 98, p. 1155.

GOTO, K. 1923 Beitrag zur Kenntnis der chemischen Wärmeregulation der Säugetiere. III. Wärmeregulation der weissen Ratte. Biochem. Zeitschr., Bd. 135, S. 107.

GREENE, J. A., AND R. P. LUCE 1931 Determination of basal metabolism of the albino rat from the insensible loss of weight. J. Nutrition, vol. 4, p. 371.

HÁRI, P. 1924 Tierische Kalorimetrie. I. Vorbesprechung. Biochem. Zeitschr., Bd. 152, S. 445.

HERZOG, D. 1930 Untersuchungen über den Grundumsatz der Vögel. Wissensch. Arch. f. Landw., Abt. B., Tierernährung u. Tierzucht, Bd. 3, S. 607.

HILL, A. V., AND A. M. HILL 1913 Calorimetrical experiments on warm-blooded animals. J. Physiol., vol. 46, p. 81.

HORST, K., L. B. MENDEL, AND F. G. BENEDICT 1930 The metabolism of the albino rat during prolonged fasting at two different environmental temperatures. J. Nutrition, vol. 3, p. 177.

———— 1934 The influence of previous exercise upon the metabolism, the rectal temperature and the body composition of the rat. J. Nutrition, vol. 7, pp. 251–276.

HOUSSAY, B. A., AND A. ARTUNDO 1929 Métabolisme du rat blanc. Compt. rend Soc. de biol., T. 100, p. 124.

JOHNSON, M. S. 1926 Activity and distribution of certain wild mice in relation to biotic communities. J. Mammalogy, vol. 7, p. 245.

KAYSER, C. 1930 Contribution à l'étude de la régulation thermique. L'émission d'eau et le rapport $H^2O : O^2$ chez quelques espèces homéothermes adultes et en cours de croissance. Annal. de Physiol., T. 6, p. 721.

KESTNER, O., C. E. JOHNSON, AND W. LAUBMANN 1931 Stoffwechsel und Sonnenstrahlung. Strahlentherapie, Bd. 41, S. 174.

MACLEOD, J. J. R. 1907 Observations on the excretion of carbon dioxide gas and the rectal temperature of rats kept in a warm atmosphere which was either very moist or very dry. Am. J. Physiol., vol. 18, p. 1.

MITCHELL, H. H., AND G. G. CARMAN 1926 Effect of excessive amounts of vitamin B on the basal metabolism of rats of different ages. Am. J. Physiol., vol. 76, p. 385.

MOLESCHOTT, J., AND S. FUBINI 1881 Ueber den Einfluss gemischten und farbigen Lichtes auf die Ausscheidung der Kohlensäure bei Thieren. Moleschott's Untersuchungen zur Naturlehre, Bd. 12, S. 266 et seq.

RICHTER, C. P. 1927 Animal behavior and internal drives. Quart. Rev. Biol., vol. 2, p. 307.

SLONAKER, J. R. 1924 The effect of pubescence, oestruation and menopause on the voluntary activity in the albino rat. Am. J. Physiol., vol. 68, p. 294.

STIER, T. J. B. 1930 'Spontaneous activity' of mice. J. Gen. Psychol., vol. 4, p. 67.

SUNDSTROEM, E. S. 1930 Contributions to tropical biochemistry and physiology. II. Supplementary experiments on rats adapted to graded levels of reduced cooling power. Univ. Calif. Pub. Physiol., vol. 7, p. 103.

SZYMANSKI, J. S. 1918 Die Verteilung der Ruhe- und Aktivitätsperioden bei weissen Ratten und Tanzmäusen. Arch. f. d. ges. Physiol., Bd. 171, S. 324.

TERROINE, E. F., AND S. TRAUTMANN 1927 Influence de la température extérieure sur la production calorique des homéothermes et loi des surfaces. Annal. de Physiol., T. 3, p. 422.

VERZÁR, F. 1929 Die Bestimmung des Grundstoffwechsels und der spezifisch-dynamischen Wirkung im Rattenversuch. Arch. f. d. ges. Physiol., Bd. 222, S. 717.

WESSON, L. G. 1931 The metabolic rate and respiratory quotients of rats following the ingestion of dextrin and during fasting. J. Nutrition, vol. 3, p. 503.

ANALYSIS OF THE COMPARATIVE FEEDING TRIAL BY THE VARIANCE AND COVARIANCE METHODS [1]

E. W. CRAMPTON

Department of Animal Husbandry, Macdonald College (McGill University), Province of Quebec, Canada

ONE FIGURE

(Received for publication June 9, 1933)

Where but a single comparison has been involved in a feeding trial, i.e., where there were but two comparative lots, statistical analysis of such data as gains, feed consumption, or gain per unit of food eaten may be made either by a consideration of the differences between pairs of replicates (Student's method) or by the analysis of variance as proposed by Fisher. In fact, if the animals were allotted by pairs, the former method may prove quicker of calculation and hence be preferred in such cases.

But whenever three or more treatments have been involved in the study, i.e. three or more comparative lots, then, providing the requirements of random sampling were satisfactorily met in the allotment and that the necessary data for the individuals comprising each of the groups were recorded, analysis by the variance method will be found more efficient, in that it affords a larger number of degrees of freedom for estimating 'experimental error.' Fisher and Wishart ('30) state: "A valid test could be made based on only one degree of freedom for errors, but . . . . the values of Z exceeded in 5 per cent or 1 per cent of trials are very large, and in conse-

[1] Contribution from the faculty of agriculture of McGill University, Macdonald College, Province of Quebec, Canada. Journal Series no. 27.

quence relatively large real effects must be judged insignificant or, in other words, escape detection. Precision begins to be lost seriously when the number of degrees of freedom for error falls below 10."

The difference in degrees of freedom available for error between ordinary feeding trials of two, three, or four lots each having ten animals per lot may be illustrated as follows:

Variance due to:	DEGREES OF FREEDOM		
	Two lots of ten animals	Three lots of ten animals	Four lots of ten animals
Between treatments (lots)	1	2	3
Between replicates	9	9	9
Error	9	18	27
Total	19	29	39

It will be seen from the above that each lot over two contributes nine additional degrees of freedom on which to estimate the error applicable to comparisons of the trial. In other words, the variability of the whole trial, less that part which can be accounted for as being due to specific and measurable factors, may be used as a basis for estimating the significance of differences between means of any two comparative lots.

The extent of this variability is best measured by the variance (s^2), for it is a characteristic of this measure that the final or total variance within a group is the sum of the variance contributed by the several independent factors causing variability.

The analysis of variance as applied to a comparative feeding trial then is simply the dividing up of the total variance of the trial into that due to each of the known causes and to that remaining or chargeable to the total of the unknown or unmeasured causes or 'experimental error.'

In every comparative feeding trial there is one cause of variability, the imposed experimental condition, and usually at least one other which is known and measurable. Thus in the statistical analysis of such trials we usually will divide the total variance into that: 1) between treatments, 2) between replicates and 3) remainder or 'error.'

Thus, if live weight gains between lots are under consideration, an analysis of the variance of these data will group under 'error' the results of all factors which have affected the live weight gains, other than those accounted for under such headings as 'between replicates.' Among such factors, the one of greatest importance in normal cases will be the amounts of feed eaten by different individual animals, and obviously the value of any study of the average gains of the groups differently treated will be enhanced if the effects of differences in quantity of feed eaten can be eliminated. The most usual method of eliminating the effect on gains of differences in feed consumption is to calculate the gain per unit of feed eaten, and to use this ratio as the basis of the analysis of the efficiency of the experimental rations in producing live weight increases.

Data from a comparative hog feeding trial recently completed at Macdonald College may be used to illustrate the methods of analysis herein discussed and table 1 gives feed consumption, live weight gains, and the calculated gain per 100 pounds of feed eaten for each pig in the aforementioned test. The totals for rows and columns are also included together with the actual and relative means for the columns.

Another method of freeing the live weight gains of the effects of varying feed consumption which may be used in trials which include data for individual food intake is by the statistical procedure known as the analysis of covariance. This method, by means of the regression of gain on feed eaten, corrects the variance of the gains to account for the effects of varying feed intake. In this way that portion of the variance in gains both between treatments and between replicates, due to difference in feed intake is eliminated. It has the advantage over the gain-feed ratio method not only of avoiding the calculation of the ratios of each animal and the errors in interpretation which may result from the use in analysis of such calculated ratios, but also of yielding at the same time separate information relative to the actual gains, the feed consumption, and the degree of correlation between them.

TABLE 1

Feed eaten, live weight gains, and gain per 100 lbs. feed eaten—data from hog feeding trial no. 33 B. Macdonald College

	LOT I			LOT II			LOT III			TOTALS OF ROWS (t_2)		
	Lbs. feed eaten (X)	Lbs. gain (Y)	Gain per 100 lbs. feed (Z)	Lbs. feed eaten (X)	Lbs. gain (Y)	Gain per 100 lbs. feed (Z)	Lbs. feed eaten (X)	Lbs. gain (Y)	Gain per 100 lbs. feed (Z)	(X)	(Y)	(Z)
	382	66	17	377	88	23	441	90	20	1200	244	60
	335	72	22	403	92	23	399	85	21	1137	249	66
	388	84	22	376	86	23	362	75	21	1126	245	66
	316	47	15	381	90	24	372	82	22	1069	219	61
	319	75	24	345	85	25	357	85	24	1021	245	73
	399	87	22	359	82	23	367	79	22	1125	248	67
	358	75	21	360	80	22	396	93	23	1114	248	66
	355	73	21	352	96	27	349	72	21	1056	241	69
	344	59	17	331	84	25	380	85	22	1055	228	64
	339	70	21	327	77	24	346	81	23	1012	228	68
Totals of columns (t_1)	3535	708	202	3611	860	239	3769	827	219	10915	2395	660
Means	353.5	70.8	20.2	361.1	86.0	23.9	376.9	82.7	21.9	363.83	79.83	22.0
Means on relative basis	94.4	88.7	91.8	99.3	107.7	108.6	103.6	103.6	99.5	100.00	100.00	100.0

A discussion of the mathematical theory behind this method is not a part of this paper. For such the reader is referred to references cited at the end of this paper. As an example of its application to the ordinary comparative feeding trial, however, the data from the same experiment involved in the analysis of variance shown in table 2 have been analyzed by the covariance method and are presented in tables 3 and 4 following.

Table 2 gives the analysis of variance for the gain per 100 lbs. of feed eaten. Comparison of the difference between the 1/2 log values for 'error' and 'treatment' (between lots) with the Z value necessary for odds of 20 to 1 shows that the differences between lots due to the experimental treatments are sufficiently greater than those expected from chance factors to justify us in claiming significantly different gains between lots per 100 pounds feed eaten.

In order to obtain a quantitative estimate of the differences between these three treatments we may, through the calculation of the standard deviation for error, determine the amount of difference which must exist between the means of any two of these lots to cover the allowance necessary for variation due to chance (or error). Such calculation indicates that some 6.6 per cent of difference is required to cover error (odds of 20 to 1) or, in other words, no differences due to treatment of less than 6.6 per cent are measurable in this trial.

In table 3 are given the analyses of variance of feed consumption (3 a), variance of gains in weight (3 b), and covariance of gains and feed consumption (3 c). The analyses in tables 3 a and 3 b in so far as the sums of squared deviations and variance (s^2) are concerned are carried out as in any ordinary analysis of variance. The calculation of the standard deviation (s) and the coefficients of variability (C.V.) are included as additional information concerning the feed consumption and gains of the animals during the trial. In table 3 c the sum of the products of gain and feed, S(XY), is obtained by procedure similar to that involved in the calculation of sums of squares for either variable (X or Y) singly,

TABLE 2

Analysis of variance of gain per 100 lbs. feed eaten (Z) from table 1

VARIANCE DUE TO	DEGREES OF FREEDOM	SUMS OF SQUARES $S(z^2)$	VARIANCE (s^2)	$1/2 \log_e s^2$
Between treatments	2	68.6	34.3	1.7676
Between replicates	9	42.7	4.7	0.7738
Error	18	62.7	3.5	0.6264
Total	29	174.0		

STANDARD DEVIATIONS AND NECESSARY DIFFERENCE	POUNDS	PER CENT
Standard deviation for error (s)	1.87	8.5
Standard deviation for single difference ($s_d = s \times \sqrt{2}$)	2.65	12.0
Standard error for difference between two means of 10 ($s_{md} = s_d/\sqrt{10}$)	0.84	3.8
[1]Necessary difference between two means of 10 ($s_{md} \times t$) with		
't' for n: 18: P = 0.05 = 2.101	1.76	8.00
P = 0.10 = 1.734	1.46	6.6

CALCULATIONS FOR OBTAINING SUMS OF SQUARED DEVIATIONS $S(z^2)$	
Total sum of squares:	
Sum of squared deviates	14694.00
Minus correction..14694.00 — (660 × 20)	174.0
Sum of squares due to treatment:	
Sum of column totals squared	145886.00
Minus correction and divided by 10 replicates [145886.00 — (660× 220)] /10	68.6
Sum of squares due to replicates:	
Sum of row totals squared	43688.00
Minus correction and divided by 3 treatments [43688.00 — (660 × 66)] /3	42.7
Z value for standard measure of significance (P = 0.05), where $n_1 = 2$; $n_2 = 18$	0.6341
Difference between 1/2 logs of variance for treatments and error (1.7676 — 0.6264)	1.1412
Differences between lots are significant.	

[1] See page 316 for definition and discussion of 'necessary difference.'

the difference being that instead of squaring the value in question (as pounds feed), the product of two corresponding values is found (as pounds feed × pounds gain). The correction for the mean is made by subtracting from the raw sum

TABLE 3

Analysis of variance and covariance of gains and feed consumption

Table 3 a. Feed eaten (X)

VARIANCE DUE TO	D/F	S(x^2)	VARIANCE (s^2)	s	GENERAL MEAN	C.V.
Between treatments	2	2853.50	1426.75			
Between replicates	9	10721.50	1191.28			
Error	18	9133.55	507.42	22.52	363.83	6.4%
Total	29	22708.55				

Table 3 b. Gains in weight (Y)

VARIANCE DUE TO	D/F	S(y^2)	VARIANCE (s^2)	s	GENERAL MEAN	C.V.
Between treatments	2	1279.27	639.63			
Between replicates	9	334.16	37.13			
Error	18	1540.72	85.60	9.25	79.83	11.46%
Total	29	3154.15				

Table 3 c. Covariance of feed eaten and gains (XY)

COVARIANCE DUE TO	D/F	S(xy)	MEAN COVARIANCE	r_{xy}	b′	b″
Between treatments	2	1143.11	571.55	0.5983	(0.4006)	(0.8936)
Between replicates	9	980.16	108.91	0.5173	(0.0914)	(2.9332)
Error	18	2832.28	157.35	0.7550	0.3101	1.8382
Total	29	4955.55			0.2182	1.5711

Sums of squares of gains corrected for feed eaten = S(y^2) — 2 (b′ for error) (Sxy) + (b′ for error)^2 S(x^2).

Sums of squares of feed corrected for gains = S(x^2) — 2 (b″ for error) (Sxy) + (b″ for error)^2 S(y^2).

of products the quantity obtained by multiplying the total of the one variable (as feed) by the mean of the other (as gain).

The mean covariance is obtained by dividing the sum of the products S(XY) by the degrees of freedom in each case. Column 5 in this table gives the correlations between gains

and feed eaten in this trial, all of which are significant. A significant value of r is of interest in showing that a portion of the variance in gains is associated with differences in feed consumption.

These values are obtained by the formula:

$$r_{xy} = \frac{\text{covariance}_{xy}}{\sqrt{s^2_x} \bullet \sqrt{s^2_y}}$$

From these data the two regression coefficients (b′, b″) were also calculated:

$$b' = \frac{\text{covariance}_{xy}}{s^2_x}$$

and

$$b'' = \frac{\text{covariance}_{xy}}{s^2_y}$$

The former gives the average number of units of change in the gains with every unit change in feed consumption, while the latter (b″) is the average change in feed consumption with each unit change in gains. It will be seen that each pound increase in feed eaten resulted in an increase af about one-third of a pound in grains. ($b' = 0.3103$).

These covariance and regression values may now be used to correct the analysis of the gains (or feed eaten) to account for differences in feed consumption (or gains). That is, by adjusting the variance of the gains for the effect of feed eaten, we obtain an analysis of gains with the effect of varying feed consumption eliminated as well as may be by a linear relationship. The formulae for calculating the corrected sums of squares are appended to table 3 and the corrected values are found in table 4. It should be noted that one more degree of freedom for error has been lost through the calculation of the constant (b) from the residuals.

Analysis of variance of gain-feed ratio vs. analysis of gains (or feed) by covariance

In the example used it will be seen that the two methods of eliminating from the live weight gains the effects of varying feed consumption, i.e. analysis of the calculated gains per 100

pounds feed eaten vs. analysis of gain and feed eaten by covariance, appear to have given approximately the same degree of precision. In table 2 it is shown that differences between gains

TABLE 4

Analysis of variance of gain and feed consumption corrected by regression (see table 3)

VARIANCE DUE TO	DEGREES OF FREEDOM	FEED EATEN—(CORRECTED FOR GAINS)			GAINS—(CORRECTED FOR FEED CONSUMPTION)		
		$S(x^2)$	s^2	$1/2 \log_e s^2$	$S(y^2)$	s^2	$1/2 \log_e s^2$
Between treatments	2	2973.60	1486.80	3.6522	844.71	422.35	3.023
Between replicates	9	8247.16	916.35	3.4102	757.27	84.14	2.216
Error	17	3927.78	231.00	2.7212	662.45	38.97	1.820
Totals	28	15147.78			2264.42		

Necessary Z for P = 0.05 where $n_1 = 2$; $n_2 = 17$ is 0.6393

	FEED EATEN		GAINS	
	Pounds	Per cent	Pounds	Per cent
Standard deviation for error	15.20	4.18	6.24	7.81
Standard deviation for single difference	21.49	5.91	8.83	11.06
Standard error for difference between means of 10	6.80	1.87	2.79	3.49
Necessary difference between means of 10:				
t (for n = 17; P = 0.05) = 2.11	14.35	3.95	5.88	7.37
t (for n = 17; P = 0.10) = 1.74	11.83	3.25	4.86	6.09

Differences

	COMPARISONS	PER CENT DIFFERENCE [1]	NECESSARY DIFFERENCE	NET DIFFERENCE	FAVOR OF
Gains	Lot I vs. II	16.15	+ 6.09	10.07	Lot II
	Lot I vs. III	5.87	± 7.37	0	..
	Lot II vs. III	10.28	+ 6.09	4.19	Lot II
Feed	Lot I vs. II	5.59	+ 3.25	2.34	Lot I
	Lot I vs. III	0.42	± 3.95	0	..
	Lot II vs. III	6.01	+3.25	2.76	Lot III

[1] Differences based on corrected lot means.

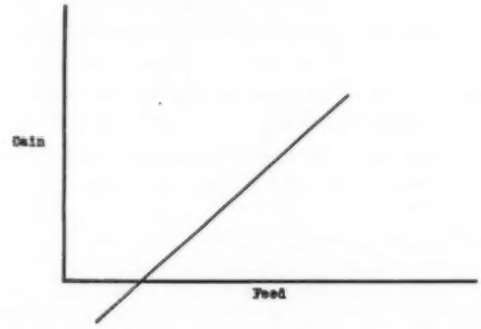
per 100 pounds feed eaten greater than 6.6 per cent can be measured and in table 4, where by covariance, effects of varying feed consumption have been corrected for, differences between gains greater than 6.09 per cent may be detected in this same trial.

TABLE 5
Summary of tables 2 and 4

METHOD	LOTS COMPARED	OBSERVED DIFFERENCE, PER CENT	NECESSARY DIFFERENCE, PER CENT	DIFFERENCE PROVED STATISTICALLY AS CREDITABLE TO EXPERIMENTAL TREATMENT
Gain per 100 lbs. feed eaten	I vs. II	16.8	6.6	10.2
	I vs. III	7.7	6.6	1.1
	II vs. III	9.1	6.6	2.5
Live weight gains corrected by covariance for varying feed consumption	I vs. II	16.2	6.1	10.1
	I vs. III	5.9	± 7.4	0.0
	II vs. III	10.3	6.1	4.2

This close agreement, however, is probably coincidental rather than to be expected, for the original calculation of the gain-feed ratio introduces a systematic error of variable magnitude which will be reflected in any analysis of the variance of this ratio. The reason for this error is that it is necessary to supply a pig with a certain amount of feed in order merely to keep it alive, without gaining in weight at all.

In other words, the line of regression of gain on feed consumed (assuming it to be linear) does not pass through the origin but looks something like this:



Consequently even if the correlation between gain and feed eaten were perfect so that the gain were uniquely defined as a linear function of the feed, that function would not be of the form $g = b\,f$, but rather $g = a + b\,f$. The gain per 100 pounds feed then becomes:

$$\frac{100\,(a + b\,f)}{f}$$

which is not a constant but a variable quantity tending to the limit b as f is increased indefinitely. As in practice f cannot be increased indefinitely, the values of the ratio obtained will be subject to systematic errors.

The extent of this error will depend upon the magnitude of the variations in feed consumption between pigs, the greater the differences in the amounts of feed eaten the greater the error, and consequently the greater the discrepancy between the results of the two methods of analysis.

The objection above raised to the gain-feed ratio as usually calculated applies particularly to its use as a basis for statistical analyses.

As a method of describing the end result of a feeding test in practical terms, the average gain made on a unit of feed (or the average feed required to produce a unit of gain) is often quite useful. When this calculation is made for each pig of the trial, however, a statistical analysis of the array of these ratios will include, in addition to the experimental error, a systematic error which may be of sufficient magnitude to seriously distort the result, and vitiate the estimate of experimental error and hence any test of significance.

The covariance method, in which the variance of the actual gains, freed from the effects of varying feed consumption by regression, is subjected to analysis, is sound both biologically and statistically. It gives the best estimate possible from the data of the significance of such differences as may exist between comparative lots. Either the gains, with the effects of varying feed consumption eliminated, or the feed eaten, with the effects of different gains made eliminated, or both, may be studied by this method. In the former case, the re-

gression of gain on feed would be used in the regression equation (b′ in table 3 c). In the latter, the regression of feed on gain would be used (b″ in table 3 c).

It should also be noted that where the estimate of error is obtained by an analysis of covariance, the differences between lot means must be based on corrected lot means. These corrected values may be determined by correcting the deviations of the lot means from the general mean for differences in feed consumption. The corrected deviations will be:

$$\overline{Y}_1 - \overline{Y} - b\ (\overline{X}_1 - \overline{X})$$

in which $\overline{Y}_1$, $\overline{Y}_2$, - - - - $\overline{Y}_n$ are the mean gains of lots 1, 2, etc.; $\overline{X}_1$, $\overline{X}_2$, - - - - $\overline{X}_n$ the mean food consumptions of lots 1, 2, etc.; $\overline{Y}$ and $\overline{X}$ the general means for gain and feed eaten respectively; and b the regression for error of X on Y or Y on X as the case may be.

It would seem, therefore, that the covariance method leads to a more desirable interpretation of the gain and feed consumption data of a feeding trial than is obtained through the analysis of gain-feed ratios calculated for the individual experimental subjects. Its use, of course, is limited to trials in which individual feed consumption is recorded.

Application to other feeding trial data. While the example here presented of the use of covariance deals with feed consumption and live weight gains, the method is equally applicable to other correlated variables. Thus in the case of trials involving successive feeding periods where the treatment during an early period may have disturbed the gains and hence the initial weights of subsequent periods, the effects of differences in initial weight, which could not be corrected for by allotment, may be eliminated from the analysis by covariance.

The necessary difference. It may be in order here to call attention to the 't' value and 'Z test' used in estimating the allowance necessary between two means for experimental error. These values change according to the number of degrees of freedom available for the estimation of the standard

errors and also with the reliability demanded. Odds of 20 to 1 or a probability of 5 per cent have recently come to be accepted as sufficient reliability for ordinary comparative feeding trials.

In testing the significance of a mean difference we are testing whether or not the observed difference deviates significantly from zero. In an analysis of variance such a test is made by comparing the Z value necessary for the significance wanted, with the Z value obtained (i.e., the difference between the 1/2 $\log_e$ values of the variances for 'between treatment' and 'error'). Differences between the 1/2 logs greater than the necessary Z value indicate that treatments have caused differences between lots greater than those chargeable to error.

The same information may be obtained from the standard deviation for error in the trial, using instead of the Z values, a a table of 't.' These values are simply the ratios which must exist between means and their standard errors for a given level of significance.

This latter is the test which must be used in comparing two means within a trial in which more than two lots are involved, for the Z test simply tells whether or not the variation between differently treated lots is significantly greater than that between animals treated alike (error). If the Z test is negative, then no further test is necessary; but if there is a significant effect of treatment indicated by this test, then the standard deviation for error is calculated (square root of the variance for error) and from it the standard error of a mean difference $\frac{s \times \sqrt{2}}{\sqrt{n}}$. In this case n is the number on which the means compared are based.

To get the necessary difference between two means the standard error of the mean difference (s_{md}) is multiplied by the appropriate 't' value, i.e. with n corresponding to the degrees of freedom from which the error of the trial is estimated and P equal to whatever odds are wanted.

$P = 0.05$ vs. $P = 0.10$ for odds of 20 to 1

The P necessary for odds of 20 to 1 will normally be at the 5 per cent point or $P = 0.05$. In cases, however, where by either the Z or t tests there has already been proved a positive or negative significant difference from zero due to treatment, the calculation of the necessary allowance for error (or necessary difference) with odds of 20 to 1 may be made using the t value given for $P = 0.10$. This is permissible since once it has been established that a significant difference of known sign exists, we need measure an allowance for error in one direction only, in which case the odds for a given P value as given in the table of t are doubled.

If 'net difference' is defined as the observed difference minus the allowance for experimental error, or the maximum difference which can be proved statistically to be due to the experimental treatment, then it follows from the above that the allowance for error used in determining the net difference will be based on the doubled P value, for unless a significant difference is established, there can be no 'net difference.' For example, the t value for $n = 17$ and $P = 0.05$ is 2.11, which in terms of difference indicates that two means of 10 in this trial must differ in gains by at least 7.37 per cent ($s_{md} \times t = 3.49 \times 2.11$) before such a difference can be considered to be due, in 95 per cent of the cases, to the experimental treatments and not to uncontrolled factors.

In comparing lots I and II, there is a difference of 16.8 per cent (based on corrected means) and if we use lot I as the base or check lot the difference is + 16.8 per cent in favor of lot II. By our test we should consider that any difference greater than + 7.37 per cent was significant. Our difference, therefore, is not only significant but positive. Had lot II been chosen as check, then the difference would have been negative.

Since we are now concerned with measuring how much the mean of lot II must be greater than that in lot I in 95 per cent of cases before we can begin to count the effects as due to feed or other experimental treatment, we may calculate

the 'net difference' by using a t value of 1.74, corresponding to $n = 17$ and $P = 0.10$.

But let us suppose that the difference between these lots had been 7.0 per cent. By our t test ($n = 17 : P = 0.05$) we should have no assurance that the true difference was 7 per cent in favor of lot II. It might just as truly have been 7 per cent in favor of lot I because by our test we have found that any difference, positive or negative, as great as 7.37 per cent might have occurred once in twenty times by chance alone. Therefore, in spite of the fact that by using the t for $P = 0.1$, we should obtain in favor of lot II a 'net difference' of + 0.91 per cent (+ 7.00 — 6.09), the figure is based on the false premise that the observed difference was in favor of lot II, and that its magnitude was 7 per cent.

This distinction between the test of significance and the calculation of a 'net difference' after the significance of the observed difference has been demonstrated, becomes of particular importance in those border-line cases where it is sometimes possible to obtain an apparent 'net difference' when actually no significant effect due to the imposed experimental conditions can be claimed. On the other hand, to forego the use of the doubled value of P when it can legitimately be applied is to be conservative to the point of materially underestimating real differences creditable to the experimental treatments under consideration.

ACKNOWLEDGMENT

The writer wishes also to acknowledge and express his appreciation of the helpful suggestions received from J. W. Hopkins, division of biology and agriculture, National Research Council of Canada.

LITERATURE CITED

FISHER, R. A., AND J. WISHART 1930 The arrangement of field experiments and the statistical reduction of the results. Imperial Bureau of Soil Science, Technical Communication no. 10, p. 12.

REFERENCES

FISHER, R. A. 1933 Statistical methods for research workers, 4th ed. Oliver & Boyd, Edinburgh.

SANDERS, H. G. 1930 A note on the value of uniformity trials for subsequent experiments. J. of Agr. Sc., vol. 20, pp. 63–73.

TIPPETT, L. H. C. 1931 Methods of statistics. Williams & Norgate, Ltd., London.

THE BIOLOGICAL ACTIVITY OF SOME CAROTENE PREPARATIONS [1]

HARRY N. HOLMES, RUTH CORBET AND HAROLD CASSIDY
Severance Chemical Laboratory, Oberlin College
AND
CLARA ROCKE MEYER AND SARAH IRENE JACOBS
Nutrition Laboratory, Department of Home Economics, College of Agriculture, University of Illinois

ONE FIGURE

(Received for publication May 29, 1933)

The possibility of feeding carotene which had been adsorbed from solution by ultra-porous powders seemed attractive. By this method expensive isolation of carotene might become unnecessary for nutrition work.

The success of such feeding must depend upon the ability of the intestinal juices to desorb or remove the carotene from the adsorbing surface of the powders. Although Marcus ('31) observed that contact with fine powders destroyed vitamin A and Lachat, Dutcher and Honeywell ('30) stated that vitamin A in cod liver oil may be adsorbed on highly activated silica gel so tenaciously that it cannot be rendered available when the silica gel is fed to rats, more encouraging results were reported by Holmes, Lava, Delfs, and Cassidy ('33). These authors were able to recover a high percentage of vitamin A adsorbed on certain porous solids by the use of proper solvents. They obtained similar results with carotene but these experiments did not prove that animals could show an effective recovery of adsorbed carotene. Hence the feeding

[1] The nutrition studies were made possible by a generous 'Grant-in-aid' from the National Research Council.

experiments described below. As porous adsorbents it was decided to use ashless norit (Miller, '26), activated alumina prepared by the Aluminum Company of America, and air-floated silica gel obtained from the Silica Gel Corporation.

Carotene and carotene-containing materials prepared in the Oberlin laboratory were sealed in glass tubes under nitrogen or carbon dioxide and received in the Illinois laboratory during the years of 1931 and 1932. The materials were fed to vitamin-A-depleted rats in order to determine to what extent the carotene was utilizable by the animal body as the sole source of vitamin A in the diet.

The preparations were stored at 1° to 10°C. from the time they were received. After the seals had once been broken, two-hole rubber stoppers fitted with stop-cock tubes were inserted into the mouths of the test tubes to facilitate maintenance of a nitrogen atmosphere during the time material was being removed for daily weighings and preparatory to continued storage under nitrogen. Commercial nitrogen was bubbled through alkaline pyrogallol to remove traces of oxygen, then through distilled water, and finally through soda lime.

EXPERIMENTAL

The curative method was employed, using young albino rats 3 to 4 weeks old of such dietary history that the first symptoms of xerophthalmia were exhibited after 21 to 28 days on the vitamin-A free regimen (Meyer and Hetler, '29). The appearance of a small line extending from the medial corner of the eye along the upper lid was found to be a reliable sign of vitamin A depletion (in no instance were the animals allowed to develop severe xerophthalmia). This sign marked the beginning of the 8 weeks' experimental feeding period. In all cases weighed amounts of the test materials were fed daily. In the later experiments, as will be indicated, the test materials immediately after weighing were placed directly into the mouth of the rats in order to minimize the possibility of the destruction of activity by exposure to air.

Careful observations were made of food intake, of weight increment, and of symptoms typical of vitamin A deficiency. Autopsies were made on all animals with special examination of the kidneys and urinary bladder for stones and of the glands at the base of the tongue for evidence of purulent material.

Several different types of control animals were used. They were as follows: negative controls, i.e., animals receiving the basal diet and no source of vitamin A; positive controls, i.e., animals reared on the same diet and cured of vitamin A deficiency symptoms by the addition of cod liver oil or pure carotene dissolved in highly purified ethyl laurate;[2] and control animals fed the ethyl laurate without the carotene.

DISCUSSION

1. In a purely preliminary series of tests the following preparations were fed: A, C, and D, with carotene adsorbed on silica, sample B with carotene adsorbed on carbon, and sample E, with carotene adsorbed on alumina. When these materials were fed to the vitamin A depleted rats for a period of 17 to 20 days in daily amounts representing a large excess of carotene, the animals were in no better nutritive condition than were the negative control rats. The xerophthalmia was not alleviated but became increasingly severe; hematuria persisted in a large number of the animals; infections of neck glands resulting in pus sacs were frequently encountered; and failure of appetite and loss of body weight always occurred.

The supplements were well eaten at first and in some cases throughout the entire test period, but in most cases they were later entirely refused. (Other control animals with severe xerophthalmia were cured within the same period with cod liver oil or crystalline carotene dissolved in ethyl laurate.) At the end of 17 to 20 days the supply of test material was exhausted partly due to the attempts to increase the dosage sufficiently to effect a cure. Cod liver oil was then given to

[2] Ethyl laurate no. 904, Research Lab. Eastman Kodak Co., Rochester, New York.

these severely deficient animals, and in every case a rapid increase in body weight resulted, although the xerophthalmia was not cured. In the thirty-four animals used kidney stones were found in two and pus sacs at the throat in three cases. The eight negative control animals from the same litters lived, as an average, 47 days after the experimental period began. Kidney and bladder stones were found in every case and pus in the glands at the base of the tongue in five of the animals. Three animals receiving cod liver oil, and seven receiving crystalline carotene in ethyl laurate after the first appearance of xerophthalmia, were of normal weight, sleek, and free from any pathological lesions at the end of the 8 weeks' test period. (Carotene was fed at levels of 0.05 mg. and 0.1 mg. daily to these animals.) No evidence could be found that any one of the samples A, B, C, D, or E supplied utilizable carotene as a source of vitamin A to the animals.

These purely preliminary experiments showed us that 0.33 mg. of carotene adsorbed (from petroleum ether) on each gram of silica gel and even as much as 0.83 mg. per gram of alumina, or 1 mg. per gram of norit carbon were not made available by the digestive juices of rats. Consequently, it was decided to increase the concentration of carotene to 3 mg. per gram of alumina. This adsorbent was selected for the final series because of its laboratory suitability for adsorption and recovery.

2. Samples H, F, and M were so prepared that 3 mg. of carotene were adsorbed on each gram of alumina. In the case of F the carotene adsorbed from petroleum ether in the presence of ethyl laurate, and of M in the presence of cholesterol.

The solvent was not quite completely removed by distillation at 50° with reduced pressure in an atmosphere of CO_2. Preliminary to adsorption the alumina, ground to 200 mesh, was activated for 3 hours in a stream of CO_2 to remove air, moisture, etc. Activation in the preliminary series was far less thorough and there may have been some loss of carotene by oxidation. Two levels of feeding were chosen in each case;

i.e., 0.08 gm. of the activated alumina carrying 0.24 mg. carotene, and 0.15 gm. of the activated alumina carrying 0.45 mg. of carotene. Immediately after the day's dose was weighed it was hand fed by means of a small spatula into the mouth of the animal. All three samples were found active at both levels fed. Figure 1 illustrates the resulting average weight increments which occurred in all cases. The negative control litter mates (twenty-one rats) were all dead before, or a few days after, the end of the 8 weeks' test period. Six animals in this series received 0.005 mg. daily of carotene dissolved in ethyl laurate. These animals did not gain as much weight as did the six animals receiving one drop of cod liver oil daily, nor was xerophthalmia as rapidly or completely cured in the carotene fed group. The growth of rats receiving the crystalline carotene in ethyl laurate was no better and in some cases not as good as that of the animals receiving test materials H, F, and M when the latter samples were used at the higher level of feeding. The nutritive condition of the animals receiving 0.15 gm. daily of test material, H, F, and M was decidedly better than that of the rats receiving the smaller amount, i.e., 0.08 gm. daily. This improvement was especially noted in the cases of test materials M and F. The animals receiving the 0.08 gm. daily of test material F were in poorest condition of all the animals (except the negative controls) but where the larger amount of test material F was fed the animals were in a better condition than were those in any of the other groups.

Activity of recovered carotene

At the two levels fed the animals were receiving approximately forty-eight and ninety times as much carotene as the animals receiving the 0.005 mg. daily dosage. It appeared that the carotene had either lost some activity during the adsorption process, or that it was only partially accessible to the animal from the adsorbents used. In order to determine whether the physiological activity of the carotene had been affected by the adsorption process, carotene which had been

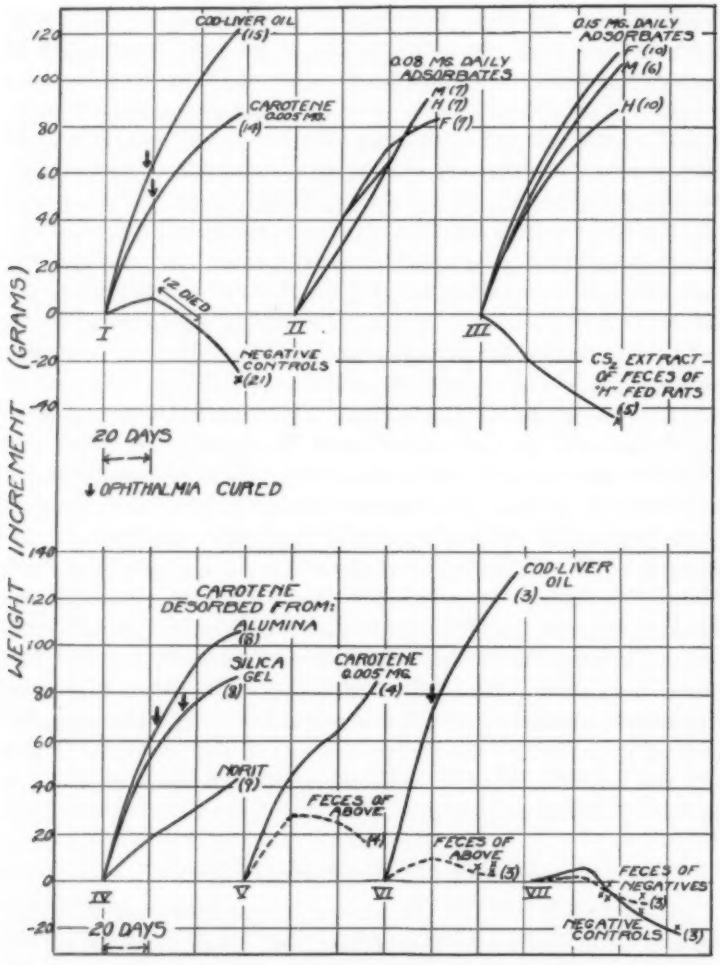


Fig. 1 Composite weight increment curves of rats receiving vitamin A free diets alone or supplemented with cod liver oil, with carotene, or with carotene preparations. Growth is plotted from the time definite xerophthalmia developed at which time the experimental test diet was begun. The number of animals represented is indicated in parentheses at the end of each curve.

I. The fifteen animals fed one drop (18 mg.) of cod liver oil daily grew at a normal rate and moderately severe xerophthalmia was entirely cured in from

desorbed or recovered from activated silica gel, alumina, and carbon was fed to other vitamin A depleted rats. The material was prepared in the Oberlin laboratory by methods previously described by Holmes, Lava, Delfs and Cassidy ('33). Carotene was adsorbed from petroleum ether and desorbed by chloroform except in the case of norit where carbon disulfide was used for recovery. Evaporation of the solvent at 50° under reduced pressure left a slight amount of solvent in the residue. In each case the recovered pigment (determined colorimetrically) was fed in ethyl laurate solution. The animals were given 0.006 mg. of recovered carotene daily and the results in average weight increment with each test material are graphically presented in figure 1, group IV. The

2 to 20 days. The fourteen animals receiving 0.005 mg. daily of carotene dissolved in pure ethyl laurate grew at a slower rate than the cod liver oil-fed rats; xerophthalmia was cured in all but three cases in from 3 to 20 days. Xerophthalmia became increasingly severe in every case where no source of vitamin A was given (negative controls). Twelve animals died 20 or 40 days after the depletion symptoms appeared and all but one were dead before the end of the 8 weeks' test period.

II and III. When carotene adsorbed on alumina alone 'H,' or on alumina in the presence of ethyl laurate 'F,' or on alumina in the presence of cholesterol 'M,' was fed at the lower level (0.08 gm. daily of material representing 0.24 mg. of carotene) growth was comparable to that of the animals receiving 0.005 mg. of carotene daily. The xerophthalmia was not as promptly cured, however, in the cases where the carotene on the adsorbate was fed even at the 0.15 mg. daily level. Growth on the higher level of feeding was somewhat improved in the cases of 'M' and 'F.'

IV. The results indicate that carotene desorbed from alumina and fed at approximately 0.006 mg. daily retains all its original activity; some activity is lost where silica gel was the adsorbent and only a small amount of activity, if any, is retained by carotene adsorbed on, and then recovered from, ashless norit. Groups V, VI, and VII show the growth response of animals receiving no source of vitamin A, or carotene, or cod liver oil as the sole sources of the vitamin. The growth response of rats receiving as the sole source of vitamin A the feces of either these same negative controls, or carotene-fed, or cod liver oil-fed animals is also shown in these three groups. Since only three of four animals have been used in this study the results may not be significant but there is indication that some carotene or vitamin A activity is lost through excretion in the feces when carotene is fed and that the vitamin A activity of cod liver oil is better utilized. The nutritive condition of the animals receiving the feces of carotene-fed rats was much better than that of the rats receiving the feces of the cod liver oil-fed animals.

carotene recovered from silica gel or from alumina cured every case of xerophthalmia. Unusually good growth and restoration of a good nutritive condition were obtained in the case where alumina had been the adsorbent. In the experiments in which norit had been used as the adsorbent xerophthalmia was never entirely cured, growth was very slow and the animals exhibited muscular incoordination throughout the entire experimental period.

The results with the alumina test material were especially interesting as they show that the physiological activity of the recovered carotene was affected little, if any, by the adsorption process. This indicated that the failure of the test materials to exhibit activity was probably due to the inability of the rat to desorb the carotene from the adsorbent. This contention was borne out by the fact that at autopsy it was observed that the fecal material about to be excreted contained a large amount of the yellow pigment.

In order to study this possibility the feces of rats fed 0.45 mg. daily of carotene adsorbed on alumina, of rats fed cod liver oil, of rats fed crystalline carotene (0.005 mg. daily in ethyl laurate) and of rats receiving no source of vitamin A (negative controls) were collected daily and in each case fed to other vitamin A depleted rats as the sole source of vitamin A. The feces of negative control animals were apparently free of any vitamin A. The three animals fed feces of the cod liver oil-fed rats were little, if any, better than the negative controls. Xerophthalmia was very severe and other infections were found on autopsy. The cod liver oil-fed animals were free from any infection and grew at a rate appreciably greater than that of any of the carotene-fed animals. There apparently was no detectable loss of vitamin A from these animals in the feces. The feces of rats fed 0.005 mg. of crystalline carotene daily, as well as of those fed carotene adsorbed an alumina apparently conveyed some vitamin A activity to the test animals to which they were fed.

SUMMARY

1. The biological activity (vitamin A) of carotene adsorbed on silica gel, on norit carbon, and on alumina has been investigated.

2. It was found that vitamin A deficient rats fed alumina which contained relatively large amounts of adsorbed carotene grew at a rate only slightly below normal but were not completely cured of xerophthalmia. However, rats similarly prepared were apparently unable to utilize adsorbed carotene when silica gel or norit carbon were the adsorbents.

3. The adsorption process did not apparently alter the physiological activity of the pigment when alumina was the adsorbate. Carotene desorbed from silica gel was somewhat less active, while carotene similarly removed after adsorption on ashless norit carbon had lost practically all biological activity.

4. Some evidence is presented which would indicate that the rat does not completely utilize all the carotene fed whether the amount given is large or small. Feces of rats fed large amounts of adsorbed carotene were highly pigmented and the feces of rats fed small amounts (0.005 mg. daily) of carotene in solution were also found to contain vitamin A activity.

LITERATURE CITED

HOLMES, H. N., V. G. LAVA, E. DELFS, AND H. G. CASSIDY 1933 Comparative studies on the adsorption behavior of crude vitamin A, carotene and cholesterol. J. Biol. Chem., vol. 99, p. 417.

LACHAT, L. L., R. A. DUTCHER, AND H. E. HONEYWELL 1930 Adsorption of vitamin A on silica gel. Penna. Agri. Expt. Sta. Bull. 258, p. 8.

MARCUS, J. K. 1931 Destructive action of finely divided solids on vitamin A. J. Biol. Chem., vol. 90, p. 507.

MEYER, C. R., AND R. A. HETLER 1929 The distribution of vitamin A in some corn-milling products. J. Agri. Res., vol. 39, p. 767.

MILLER, E. J. 1926 Adsorption from solution by ash-free adsorbent charcoals. I. A method for the purification of adsorbent charcoals. J. Physical Chem., vol. 30, p. 1031.

HUMAN MILK STUDIES

XIII. VITAMIN POTENCY AS INFLUENCED BY SUPPLEMENTING THE MATERNAL DIET WITH VITAMIN A [1]

SYLVIA SCHIMMEL McCOSH, ICIE G. MACY, HELEN A. HUNSCHER, BETTY NIMS ERICKSON AND EVA DONELSON

The Research Laboratory [2] of the Children's Fund of Michigan, and the Children's Hospital of Michigan, Detroit

(Received for publication May 18, 1933)

Animal experimentation has indicated that the concentration of vitamin A in milk is dependent upon its presence in the diet (McCollum, Simmonds and Pitz, '16; Kennedy and Dutcher, '22; Nelson, Lamb, and Heller, '22). Kennedy, Palmer, and Schlutz ('23) reported that 10 cc. of breast milk daily from women on an adequate dietary met the vitamin A requirements of rats, but that milk from women on diets deficient in this substance did not. In this laboratory daily intakes of 2.5 and 3 cc. of pooled milk from a group of wet nurses on average American dietaries were found to satisfy the vitamin A needs of both growth and reproduction of the rat (Macy et al., '27). Conversely, similar quantities of milks of two women with high production records tested singly in a similar manner contained a lower amount of vitamin A, although the maternal diets were apparently satisfactory (Macy et al., '28).

These observations on the biological potency of breast milk become more significant when considered in the light of the recent study of Thatcher and Sure ('32) in which they report

[1] This study was kindly aided by a grant from the Committee on Scientific Research of the American Medical Association.

[2] Formerly the Nutrition Research Laboratories of the Merrill-Palmer School and the Children's Hospital of Michigan.

autopsy findings of a case of avitaminosis in an infant who had been fed exclusively by breast milk from a starving mother. That the infant had been suffering from a lack of vitamin A in the diet before its admittance to the hospital was demonstrated by the presence of bilateral ophthalmitis with a left corneal ulcer 5 mm. in diameter, the outward manifestation of an acute nutritional upset which has been present for some time, and in spite of the administration of a diet containing cod liver oil during the last 14 days of life death occurred from bronchopneumonia. Post-mortem findings were those typical of vitamin A deficiency.

In view of the variability in the vitamin A concentration of mother's milk and its dependence upon her diet and subsequent availability of vitamin A from her body tissues and through breast milk to satisfy the particular physiological needs of growth of the infant, it is of clinical significance to determine whether breast milk can be enriched by supplementary additions of the maternal dietary with a concentrated source of this substance. For this purpose three women each producing large but different quantities of milk expressed all of their milk daily and besides feeding their babies by bottle were able to send sufficient milk to the laboratory for the biological tests.

The pre-cod liver oil studies on the vitamin A content of the separate milks produced while the three women were on their accustomed home diets were begun 6 weeks post-partum after mature milk flow had been established and extended to the sixth month. After these preliminary tests were complete each woman then took daily 15 gm. of cod liver oil[3] in gelatin capsules for 1 month preceding and throughout the seventh to the ninth months of observation to determine if vitamin A is transferred quantitatively into the milk from the dietary adjuvants. The quantity of milk secreted daily by each woman remained practically constant throughout the period of observation, namely, 3134, 2366, and 1419 cc. daily

[3] We wish to express our thanks and appreciation to A. D. Emmett, Ph.D., and E. A. Sharpe, M. D., of Parke, Davis & Company, Detroit, for the cod liver oil used in this study.

with average fat contents during the former period of 3.9, 3.8, and 4.8 gm. per 100 cc., respectively and during the period of cod liver oil consumption of 4.0, 3.6, and 5.5, respectively (Nims et al., '32). The breasts were completely stripped each morning, the milk being allowed to flow directly into a glass bottle, then sealed air tight, and packed in ice until it was fed 2 hours later to the test animals. A technic previously standardized for these studies and reported elsewhere (Macy et al., '27) was used for the biological testing of the milk. Quantities of ½, 1, 1½, 2, and 2½ cc. of each milk were fed daily to the experimental animals apart from the basal diet as the sole source of vitamin A. Weekly body weight and food consumption records were made. The results were recorded in average gain of all animals receiving a particular quantity of an individual milk. In addition, the time of the opening of the vaginal orifice was noted and vaginal smears were examined daily thereafter for the detection of persistent cornified epithelial cells, one of the earliest symptoms of vitamin A exhaustion in the body (Evans and Bishop, '22).

From the average gain of groups of test rats fed quantities of ½, 1, 1½, 2, and 2½ cc. daily of the individual breast milks as the only source of vitamin A it was evident that as the quantities of each milk were increased the growth and physiological well-being of the experimental animals responded proportionately. One-half cubic centimeter of milk daily was insufficient for satisfactory growth, but as the quantities were augmented improvement occurred with each succeeding increase until nearly normal growth with the entire disappearance of all symptoms of avitaminosis and complete return of the normal oestrous cycle was secured with 2½ cc. of milk daily. The total food intake of rats expressed in calories per square meter of surface area per 24 hours was not a determinate factor. As judged by the average gain in body weight of the experimental rats and the vaginal smear records, the vitamin A content of the individual milks from the three women irrespective of volume flow of milk and fat contents was essentially the same and remained unchanged during the

period of supplementary additions of 15 gm. of cod liver oil daily to an abundant and well-chosen dietary.

These findings recorded on the inability of three women to transfer increased quantities of vitamin A to their milk beyond the usual potency on an adequate dietary when daily supplementary additions of vitamin A were made to the maternal diets are in accord with those of Moore ('31, '32) who found that in the cow the vitamin A and carotene of the butterfat are maintained at normal values in spite of large dietary excess. Dann ('32) made a similar observation on rats and rabbits, stating, "There must be some factor at work limiting quite strictly the amount of vitamin A which can pass through the placental barrier or into the milk." Of additional interest is the study of Vogt ('32) who found that the vitamin A content of tissues obtained from women at operation show that the subcutaneous fat tissue is an important place of storage of vitamin A, the concentration being greatest in the mammary gland. The parenchyma of the mammary gland itself may have the function of controlling the maximum amount of vitamin A that passes into the milk.

These studies on the transmission of vitamin A are paralleled by similar ones on vitamin B. Gunderson and Steenbock ('32) found that increasing vitamin B intake both absolutely and in relation to the amount of milk secreted had no discernible effect on the vitamin B content of cow's and goat's milk and they suggest that the maximum vitamin B content is under definite physiological control. Observations on women in this laboratory corroborate the above findings (McCosh et al., '31; Donelson and Macy, '34). In studying milks of different women before and after supplementing their usual diets with 10 gm. of yeast daily it was found under the conditions of the experiments that the vitamin G potency of the breast milk was increased but there was no appreciable change in the vitamin B concentration.

In face of the fact that mother's milk produced during inadequate dietaries may fall short of meeting the optimal concentration of vitamin A in the milk and infants may fail

to secure adequate amounts of this factor for optimal growth and storage, clinicians can well afford to see that their nursing mothers receive in their diets a sufficient amount of this important food substance to maintain its maximum values in breast milk and thereby benefit both the mother and her child.

SUMMARY

The vitamin A content of the individual milks for three women remained essentially the same before and during a period of supplementary additions of 15 gm. of cod liver oil daily to an abundant and well-chosen diet.

The results of the biological assays are based upon the average gain in body weight of the experimental rat and on the vaginal smear records. It is pointed out that mother's milk, produced during an inadequate dietary regime, may fall short of meeting the optimal concentration of vitamin A in the milk and therefore the physiological needs of the infant.

LITERATURE CITED

DANN, W. G. 1932 The transmission of vitamin A from parents to young in mammals. J. Biochem., vol. 26, p. 1072.

DONELSON, E., AND I. G. MACY 1934 Human milk studies. XII. The vitamin B and vitamin G content before and during maternal consumption of yeast. J. Nutrition, vol. 7, p. 231.

EVANS, H. M., AND K. S. BISHOP 1922 On the relations between fertility and nutrition. II. The ovulation rhythm in the rat on inadequate nutritional regimes. J. Met. Research, vol. 1, p. 335.

GUNDERSON, F. L., AND H. STEENBOCK 1932 Is the vitamin B content of milk under physiological control? J. Nutrition, vol. 5, p. 199.

KENNEDY, C., AND R. A. DUTCHER 1922 Vitamin studies. IX. The influence of the diet of the cow upon the quantity of vitamines A and B in the milk. J. Biol. Chem., vol. 50, p. 339.

KENNEDY, C., L. S. PALMER, AND F. W. SCHLUTZ 1923 The vitamin content of breast milk. Tr. Am. Pediat. Soc., vol. 35, p. 26.

MACY, I. G., J. OUTHOUSE, A. GRAHAM, AND M. L. LONG 1927 Human milk studies. II. The quantitative estimation of vitamin A. J. Biol. Chem., vol. 73, p. 175.

MACY, I. G., J. OUTHOUSE, AND H. A. HUNSCHER 1928 The variability in vitamin content of human milks. J. Home Economics, vol. 20, p. 897.

MACY, I. G., J. OUTHOUSE, M. L. LONG AND A. GRAHAM 1927 Human milk studies. I. Technique employed in vitamin studies. J. Biol. Chem., vol. 63, p. 153.

McCollum, E. V., N. Simmonds, and W. Pitz 1916 The relation of the unidentified dietary factors, the fat-soluble A, and water-soluble B, of the diet of the growth-promoting properties of milk. J Biol. Chem., vol. 27, p. 33.

McCosh, S. S., I. G. Macy, and H. A. Hunscher 1931 Human milk studies. VI. Vitamin potency as influenced by supplementing the maternal diet with yeast. J. Biol. Chem., vol. 90, p. 1.

Moore, T. 1931 Vitamin A and carotene. VII. The distribution of vitamin A and carotene in the body of the rat. J. Biochem., vol. 25, p. 275.

———— 1932 IX. Notes on the conversion of carotene to vitamin A in the cow. J. Biochem., vol. 26, p. 1.

Nelson, V. E., A. R. Lamb, and V. G. Heller 1922 The vitamin requirement of various species of animals. III. The production and cure of xerophthalmia in the suckling. Am. J. Dis. Child., vol. 23, p. 518.

Nims, B., I. G. Macy, H. A. Hunscher, and M. Brown 1932 Human milk studies. X. Daily and monthly variations in milk components as observed in two successive lactation periods. Am. J. Dis. Child., vol. 43, p. 1062.

Thatcher, H. S., and B. Sure 1932 Avitaminosis. III. Pathologic changes in tissues of the albino rat during early stages of vitamin A deficiency. Arch. Path., vol. 13, p. 757.

Vogt, E. 1932 Ueber die Vitaminbestände des weiblichen Organismus. Münch. med. Wochschr., Bd. 79, S. 1570.

ANTERIOR PITUITARY GROWTH HORMONE AND THE COMPOSITION OF GROWTH

MILTON O. LEE AND NORWOOD K. SCHAFFER

The Memorial Foundation for Neuro-Endocrine Research, Harvard Medical School, Boston, Massachusetts

ONE FIGURE

(Received for publication May 31, 1933)

One of the steps to be taken in the study of the physiologic mechanisms through which the growth hormone of the anterior pituitary acts is the determination of the character and composition of the growth which is effected. If the increase in weight commonly obtained by the injection of potent extracts is due merely to the addition of water or fat, it can hardly be regarded as true growth, which must be considered to involve essentially an increase in the mass of body protoplasm, with protein synthesis a necessary accompaniment. Between the extremes of dwarfism produced by removal or destruction of the anterior lobe and giantism produced by hyperactivity, there is no question that true growth in the latter sense is stimulated or conditioned by the hormone. The stimulation, however, may involve neither a general effect upon anabolic processes, nor any specific effects upon metabolic mechanisms of the body for the synthesis and deposition of particular constituents. It may be facilitated by or entirely dependent upon such factors as appetite, digestibility and the amount of food consumed, and act by causing a plethora of nutritive elements to be available for ordinary processes. That these factors are of real significance is indicated, among other things, by some pertinent evidence of Osborne and Mendel and their associates. For example,

Anderson and Smith ('32) have been able to produce giant rats, rivaling in size those obtained by treatment with anterior pituitary extracts or grafts, merely by the feeding of particularly favorable diets.

If the excess growth caused by the hormone is partially or wholly independent of these factors and is not solely due to the character of the diet or to an increased food consumption then it is reasonable to look for specific influences upon particular metabolic mechanisms or for some property of the principle necessary for the anabolic use of the available food constituents.

In the present investigation we have determined the effects of the growth hormone upon the gain in weight and upon the composition of that gain in rats, with the factor of the amount of food consumption carefully controlled. In order to realize this control, the 'paired-feeding' method, as highly developed and analyzed by Mitchell ('30) was chosen. This method would appear to be peculiarly suitable for studies of endocrine effects upon nutrition. It is not necessary to feed rather artificial purified diets from which certain constituents under study have been carefully excluded and upon which the best growth obtainable may not be very great. On the contrary, complete diets adequate for normal growth or better, can be used advantageously, provided they do not contain in an assimilable form the hormone under study.

EXPERIMENTAL PROCEDURES

The rats were selected as pairs, triplets or quadruplets. In each set they were litter-mates of the same sex and of nearly the same body weight and body length at the beginning of the experiment. Upon unrestricted feeding they had also demonstrated equal abilities for growth since weaning. In addition, they were so distributed in the two experimental groups as to give those which were to serve as controls whatever slight initial advantage there was in individual weight, length and past growth over those which were to receive the growth hormone. The ages ranged from 52 to 224 days.

One animal of each pair, trio or quartet was chosen for a control and a mate to be treated. The third rat of each trio, designated as a 'check,' was killed at the beginning of the experimental period. Its analyzed composition was assumed to be the composition of its two mates at that time. Of each quartet, one animal became a control, one a treated, one a check and the fourth was chosen to be a treated rat fed ad libitum. There were two sets of pairs, eight sets of triplets and two sets of quadruplets. The check rats and their control and treated litter-mates are correspondingly numbered in the tables. In addition, check rat 6 was the trio mate of pair 11, and check rat 7 was the trio mate of pair 12. Two pairs, 9 and 10, had no check mates for analysis; their initial composition was calculated from the average composition of check rats 2, 3, 4, 5 and 6. The two treated rats fed ad libitum were included in order to determine directly the influence of the hormone upon absolute appetite and the maximal growth which could be secured with conditions the same except as to the amount of the food consumption.

In figure 1, B and C are the composite growth curves of ten of the rats selected for treatment and their ten control mates for 7 weeks before the beginning of the experimental period. Feeding during this time had been unrestricted and the two groups had maintained essentially the same growth rates, with those destined to be the controls a few grams heavier each week. Two pairs of animals were too young to be included in these curves. The composite growth curve of seven of the set mates selected for carcass analysis at the beginning of the experiment is shown in A.

The diet used commonly gives growth rates exceeding those of Donaldson and of King ('24). Its composition was: whole ground wheat, 49.7 per cent; yellow corn meal, 6.0 per cent; G.L.F. calf meal, 7.0 per cent; casein, 12.0 per cent; skim milk powder, 16.0 per cent; fat (lard, butter, corn oil, cod liver oil concentrate), 4.0 per cent; salt mixture (Hawk's modification of Osborne and Mendel's), 4.3 per cent; Harris yeast concentrate, 'Bemax' and lemon juice, 1.0 per cent. The food was sifted, the screenings reground and the whole batch

thoroughly mixed in a Hobart machine. The mixed food was sealed in 1-pound cans until needed. Analyses of the food

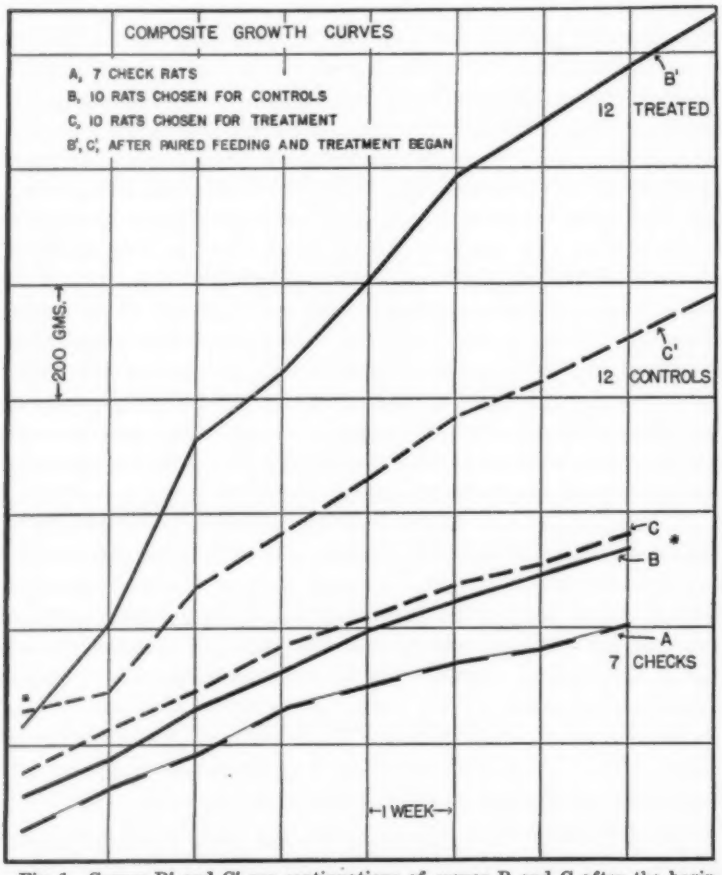


Fig. 1 Curves B' and C' are continuations of curves B and C after the beginning of paired-feeding and treatment, and with the addition of two pairs of animals.

showed its composition to be: water, 8.4 per cent; fat, 5.6 per cent; ash, 6.4 per cent; nitrogen, 3.7 per cent; carbohydrate (by difference), 56.5 per cent; and energy value, 4.20 Calories per gram. The food was fed in 35 cc. porcelain evaporating

dishes inserted into receptacles attached to the outside of the cages. These receptacles were made from 8-ounce ether cans, provided with a metal apron which covered about one-fourth of the food dish and which extended about 3 cm. inside the cage. The opening was of such size and the apron was at such an angle that a rat could get its head but not its feet into the food dish. As further precaution against wastage the food was moistened to a thick paste with water. Particular search was made each day for uneaten food carried out into the cage, and on only one occasion was any evidence found of such wastage. The rats were confined in individual wire cages 12 inches in diameter and 6 inches high with screen bottoms.

At each daily feeding the treated and control mates of each pair were given the same quantity of food. This amount was so adjusted that the less hungry member of the pair usually left about 1 gm. of its ration uneaten. Each animal's food was weighed daily to 100 mg. from its individual weighed flask. On the last day of each week the amount fed was diminished a few grams so that even the less hungry mate would consume all that was offered, and the total intake for the week was equalized by weighing the individual flasks to 5 mg. The amount of food eaten by each group of twelve animals was 9766 gm. in the whole period of 763 days, or an average of 12.8 gm. per rat per day. Water was allowed at will, but for eight pairs the amounts consumed weekly were measured. The length of the experimental period of paired-feeding and treatment before killing for carcass analyses was 8 weeks for five pairs, 9 weeks for three pairs, 10 weeks for two pairs and 11 weeks for two pairs.

For eight pairs of animals and two ad libitum feeders, collections of urine, feces and shed hair were made. For this the cages were set into paraffin-coated tinned funnels with removable brass-wire screens. Feces were removed daily and the whole cage bottom and funnel thoroughly washed down with hot ½ per cent acetic or hydrochloric acid. The use of a spray gun permitted very thorough washing without diluting the urine too greatly. The urine collections were pooled weekly for each group of rats, and samples were preserved in an ice box with small amounts of chlor-thymol. Feces were collected for the whole period, dried to constant weight at 60°C., finely ground, thoroughly mixed, and again dried. The shed hair and the alimentary tract contents at the time of killing were collected, weighed, dried and analyzed separately.

Standard procedures and methods for chemical analyses were used. Each rat was killed with illuminating gas, and the alimentary tract was stripped of its contents. The body, including the emptied gut (hereafter called the 'empty carcass' and its weight 'E. C. W.') was ground and dried to constant weight at 60°C. Fat of the hashed and dried empty carcass was thoroughly extracted with petroleum ether in a Soxhlet apparatus. The fat-free dry tissue was pulverized, mixed and dried again. Samples of this powdered tissue were taken for nitrogen, ash and calorific value determinations. The ether extract after evaporation of the ether, was analyzed for the small amount of nitrogen and ash which it contained, and its heat of combustion was determined. For nitrogen, the macro-Kjeldahl method was used; for ash, incineration in a muffle furnace; for heat value, samples were burned in the oxycalorimeter of Benedict and Fox ('25). The same methods were used for the analyses of urine, feces, shed hair, alimentary tract contents and food, except that for the calorific value of urine the figure 8.09 Calories per gram of nitrogen was assumed (Benedict and Milner, '07). The determinations on ash are the least accurate of the analyses. The average difference in duplicate ash determinations on all tissue samples was 2 per cent, and the absolute values for tissues are somewhat low because of the method of incineration used.

The anterior lobe extract used was made by ourselves from fresh beef pituitary glands.[1] It was a purified, assayed preparation, highly potent in stimulating growth in either hypophysectomized or normal assay rats. The amount injected subcutaneously each day corresponded to three rat units by our assay (unpublished results), or between one-half and three-fourths of the least amount which will cause approximately maximal growth response in rats under the assay conditions. The amount corresponded also to the growth-promoting potency of from 40 to 60 mg. of fresh beef anterior lobe. A similar but less purified extract of ours was found by Riddle ('32) with his highly sensitive pigeon test to be free of the lactation principle and to have only traces of the gonad-stimulating principle. By the less sensitive rat test

[1] We are indebted to Mr. Frederick Fenger and Armour & Co., Chicago, for supplying us with fresh pituitary glands, shipped packed in CO_2 snow.

and much larger doses of the extract used in these experiments we could not detect any gonad-stimulating activity.

RESULTS

Weight. As shown by the composite growth curves B′ and C′ in figure 1, the twelve treated animals immediately passed their control mates in weight after paired-feeding and treatment with the hormone were begun, and continued to show an

TABLE 1

Weekly excess gains (in grams) of treated rats over controls

PAIR NO.	WEEKS											TOTAL
	1	2	3	4	5	6	7	8	9	10	11	
1F	8	12	8	12	3	7	11	−4	8	10		75
2F	14	11	−2	12	8	4	0	1	0			48
3F	15	19	5	0	3	3	−7	2				40
4F	20	19	6	7	4	5	8	−2	4			71
5F	19	16	9	7	8	8	0	−2				65
6F	13	14	−4	11	3	10	−3	−4				40
7M	−3	−5	−3	5	0	−6	−7	4	2	−3	6	−10
8M	8	−9	6	14	9	5	30	3	0			66
9F	9	14	1	8	14	5	6	5	−10	3		55
10F	21	6	10	−5	5	4	−4	−3				34
11F	15	15	0	11	2	6	0	2				51
12M	4	1	3	−5	5	−10	−4	7	5	0	2	8
Totals	143	113	39	77	64	41	30	9	9	10	8	543
Ad lib. feeders 2AF, 3AF	40	33	27	9	19	6	7	7				148

excess gain each week to the end of the experiment. The curves in figure 1 are plotted only to the eighth week since at that time five pairs were killed for analysis.

The distribution of the excess gains of the treated rats by weeks and by pairs is given in table 1. At the end of the whole period eleven of the twelve had shown an excess gain in weight over their control mates on the same food intake, amounting to 543 gm., or equivalent to the weight of two extra adult animals. The means of the excess gains by the treated

animals were 5.0 gm. per rat per week and 45.3 gm. per rat for the whole period. On the assumption that, if the growth hormone were without effect, the chances are even that either rat of any pair would gain more than its mate, the observed outcome favoring the treated animal in eleven of twelve instances would be expected to occur by chance once in 341 times. With a probability of this order it is obviously unnecessary to carry the statistical analysis further, either by consideration of the magnitude of the total excess gains or of the 109 weekly comparisons of individual gains.

Treated rat, 7TM, which gained 10 gm. less than its control mate and another rat, 12TM, which showed only 8 gm. excess gain, were males from the same litter and were only 52 days old when the experiment began. They failed to show any excess gain from the first, but they and their controls were already growing rapidly. At the time they were killed their growth rates were still high and they were perhaps just approaching their plateaus. The other male rat, 8TM, did not show much excess gain at first, but apparently became more responsive as the treatment continued. That male rats are relatively more refractory to the growth hormone has been noted by Evans and Simpson ('31), and occasionally a whole litter, both males and females may be partially resistant. Rat 7TM, despite its negative response as judged by body weight, showed a positive effect in the composition of its gain. It added 1.1 gm. of nitrogen, 4 gm. of water and 0.9 gm. of ash more than did its control mate, and 10.7 gm. less of fat. Rat 12TM showed as anomalous a response in its composition as in its weight. It gained an insignificant excess of nitrogen, 0.5 gm., the same amount of ash and 10 gm. more of fat. Its excess gain in body length, 0.9 cm., is probably significant.

Notwithstanding the dilution which the inclusion of these two young males gives, the effect of the hormone on the gain in weight of the treated group as a whole was considerable. These twelve animals gained 1295 gm., or 55 per cent, of their initial live weight. Their twelve controls gained 764 gm., or 32 per cent, of their initial weight.

Body length, nutritive ratio and surface area. The average percentage increase in body length was 7.4 for the control and 13.5 for the treated rats. For each group this was only about one-fourth as much as the average percentage increase in weight. This, however, does not indicate the development of any abnormalities in body shape. Even the excess growth of the treated animals was not disproportional, as indicated by their nutritive ratios. This ratio $\frac{W^{1/3}}{L}$, gives a good measure of body shape and reflects disproportions between weight and length. The average ratios for the check, and for the control and treated rats at the beginning were 0.284, 0.283 and 0.283, respectively, with standard deviations of ± 0.004. For both the control and treated rats at the end of the experiment, the average ratio was 0.290 (S.D. ± 0.005). Such an increase in the ratio is about what would be expected in normal well-nourished rats passing the plateaus of their growth curves, and it is only surprising that the more rapidly growing treated animals did not show a greater elongation of the trunk relative to the linear dimension of body weight than did the controls.

The sums of the surface areas, calculated from body weights and lengths (Lee and Clark, '29) at the beginning of the experiment were 3528 sq.cm. for the twelve controls and 3517 sq.cm. for the twelve treated. The controls gained a total of 562 sq.cm., or 16 per cent, and the treated 991 sq.cm., or 28 per cent, an excess over the controls of 429 sq.cm., or 12 per cent, of their initial area.

Appetite. An opportunity was afforded by the technic of the paired feeding method to compare the relative appetites of control and treated rats. The results were usually indeterminate for the last day of each feeding week because that day's ration was cut for each rat so that even the less hungry member would eat all of its allowance. If both rats of a pair left approximately the same amounts of food they were considered to be equally hungry.

The appetite of each rat was determined on unrestricted feeding for a few days before the beginning of the paired-

feeding. Of 30 such determinations the rats destined to be controls were more hungry 18 times and those to receive treatment 10 times. This might be expected from the fact that the controls were slightly heavier than their mates. During the course of the experiment in 763 daily instances, the control rats were more hungry 91 times, 50 of which were in the first week; the treated rats were more hungry 555 times, 31 of which were in the first week; both rats were equally hungry 10 times; and in 107 instances the result was indeterminate. Hence, after the first week, in only 46 of 575 determined instances were the controls more hungry than the treated.

The conclusion is confirmed by direct observation and by the food consumption of the two rats fed ad libitum. The treated rats ate the most of their daily food allowance soon after it was supplied to them. Often the entire amount was eaten within 3 to 4 hours. The controls, on the other hand, usually did not consume much or any of their food until night. The amounts consumed by the two treated rats fed ad libitum were 824 and 741 gm. in the 63 and 56 days they were on the experiment, or 13.2 gm. per rat per day. This was an average excess of 1.6 gm. per day, or 13 per cent, over their mates on paired-feeding.

Water consumption. The water intake was not restricted, but the amount that was drunk was measured for the eight control and eight treated animals used for urine and feces collections. Each of these groups in 504 days drank practically the same amounts, 5.6 kg. This was in addition to the approximately equal amounts they received in the moistened food.

Activity and behavior. Unfortunately, no quantitative records could be made of the voluntary muscular activity of the animals. Incidental observations, however, gave the impression that the treated animals were more alert and active than their controls. The consumption of much of their daily rations immediately after feeding has been mentioned. They also usually cleaned their food dishes thoroughly, whereas the

controls commonly left dried particles adhering to the edges where access was difficult. The controls usually slept the greater part of the day, but the treated rats were easily aroused to such activities as gnawing at the wire cages and the metal food-dish holders. They were decidedly less tame to handle than the controls.

Composition of the animals and of the gains. The compositions of the individual check rats are given in table 2, and of the treated and controls, determined at the end of the experimental period, in table 3. Several considerations indicate the validity of the assumption that the initial compositions of the two groups on paired-feeding can be estimated

TABLE 2

Data on check rats, killed at beginning of experiment, and composition of carcasses without alimentary tract contents

NO. AND SEX	AGE	BODY LENGTH	LIVE WEIGHT	EMPTY CARCASS WEIGHT	COMPOSITION, BY WEIGHT AND PERCENTAGE, OF EMPTY CARCASS				ENERGY
	Days	Cm.	Gm.	Gm.	Water	Ether ext.	Total N.	Ash	Cal./gm. E.C.W.
1ChF	101	18.6	143	137.7	85.1 61.8%	18.0 13.1%	4.32 3.14%	5.99 4.35%	2.35
2ChF	101	19.4	158	150.7	94.5 62.7%	16.1 10.7%	5.00 3.32%	7.42 4.92%	2.20
3ChF	153	20.3	177	165.5	105.9 64.0%	13.3 8.0%	5.84 3.53%	7.95 4.80%	2.01
4ChF	107	20.4	207	197.1	116.8 59.3%	32.9 16.7%	6.19 3.14%	7.89 4.00%	2.65
5ChF	159	21.6	250	244.5	142.8 58.4%	43.9 18.0%	7.61 3.11%	10.45 4.27%	2.75
6ChF	171	21.0	206	201.3	122.6 60.9%	27.4 13.6%	6.56 3.26%	8.92 4.43%	2.43
7ChM	53	19.0	172	155.4	104.6 67.3%	12.6 8.1%	4.96 3.19%	5.85 3.76%	1.87
8ChM	224	23.4	293	280.7	174.7 62.2%	31.0 11.1%	9.87 3.52%	11.60 4.13%	2.24
Totals	1069	163.7	1606	1533.0	947.0 61.8%	195.3 12.8%	50.35 3.28%	66.07 4.31%	2.34

TABLE 3

Data on control and treated rats at beginning and end of experiment, and analyzed composition of empty carcasses at end

NO. AND SEX	AGE, DAYS		BODY LENGTH, CM.		LIVE WEIGHT, GM.		EMPTY CARCASS WEIGHT, GM.	COMPOSITION OF EMPTY CARCASS AT END				
	Begin	End	Begin	End	Begin	End		Water, gm.	Fat, gm.	Nitrogen, gm.	Ash, gm.	ENERGY, Cal.
1CF	101	171	18.5	19.7	143	188	180	105	33.6	5.37	7.6	502
1TF	101	171	18.6	22.2	142	262	250	165	21.7	8.23	10.2	489
2CF	101	164	19.3	20.5	153	202	193	111	36.8	5.96	8.2	550
2TF	101	164	19.2	21.3	156	252	241	140	44.4	7.59	9.2	673
3CF	153	209	20.2	20.8	183	211	200	114	37.4	6.43	9.3	572
3TF	153	209	20.4	22.1	186	250	236	135	41.7	7.50	10.5	659
4CT	107	170	20.2	21.9	200	247	236	130	52.3	7.19	9.8	737
4TF	107	170	20.3	23.1	195	312	295	186	34.6	9.70	12.9	659
5CF	159	215	21.9	22.6	252	282	267	147	58.3	8.07	11.2	828
5TF	159	215	21.8	24.0	250	344	320	198	40.8	10.62	13.3	749
6CF	171	227	21.0	21.3	209	222	208	122	35.1	6.74	9.0	563
6TF	171	227	20.9	22.0	205	256	239	150	26.8	7.73	11.1	534
7CM	52	129	19.0	24.2	166	373	354	212	71.8	8.89	11.6	991
7TM	52	129	18.9	24.2	168	364	356	217	61.3	10.04	12.5	925
8CM	224	287	23.4	23.8	290	324	305	188	44.1	9.78	12.8	748
8TM	224	287	23.6	25.8	290	390	369	241	35.9	12.06	14.6	753
9CF	145	215	21.0	21.3	230	273	265	143	68.4	6.83	9.0	885
9TF	145	215	21.2	23.3	228	325	314	204	34.1	9.69	13.5	664
10CF	135	191	20.8	21.3	207	228	220	123	44.4	6.76	9.9	655
10TF	135	191	20.9	22.9	203	258	248	160	22.5	8.75	11.0	509
11CF	171	227	21.0	21.3	209	220	213	122	37.0	6.79	9.3	588
11TF	171	227	20.8	22.1	201	264	248	161	22.3	8.35	10.5	504
12CM	52	129	18.6	24.4	140	376	363	227	52.5	11.01	13.2	872
12TM	52	129	18.2	24.9	134	376	365	217	62.7	11.30	13.0	973
Totals control	1571	2334	245	263	2382	3146	3004	1743	572	89.8	120.8	8490
Totals treated	1571	2334	245	278	2358	3653	3481	2175	449	111.6	142.2	8091

with a sufficient degree of accuracy from the analyses of their check mates. The only limitation of the assumption is the normal variation, which should be at a minimum among litter-mate rats of the same sex, weight and previous growth history. Although enough data are not available to give a statistical assessment of these limits, the analyses published by Mitchell and Carman ('26) indicate that the variability is less for litter-mates than for a random selection of rats of about the same age. The litter-mates of their series, how-

TABLE 4

Compositions of check, control and treated rats

GROUP	EMPTY CARCASS WEIGHT, GM.	AVERAGE COMPOSITION OF EMPTY CARCASS					
		Water	Ether ext.	Fat-free dry tissue	Nitrogen	Ash	Energy Cal./gm.
12 checks	2273	62.0%	12.7%	25.3%	3.25%	4.29%	2.33
S.D. ±		2.64	3.14	1.25	0.147	0.353	0.267
P.E. ±		0.51	0.61	0.24	0.029	0.069	0.052
C.V. ±		4.4%	24.8%	4.9%	4.5%	8.2%	11.4%
12 controls	3004	58.0%	19.1%	22.9%	2.99%	4.02%	2.83
S.D. ±		2.54	3.10	1.57	0.225	0.420	0.257
P.E. ±		0.50	0.60	0.31	0.044	0.082	0.050
C.V. ±		4.4%	16.3%	6.8%	7.4%	10.4%	9.1%
12 treated	3481	62.5%	12.9%	24.6%	3.20%	4.09%	2.32
S.D. ±		2.80	3.61	1.33	0.168	0.337	0.301
P.E. ±		0.54	0.70	0.26	0.033	0.066	0.059
C.V. ±		4.4%	28.0%	5.4%	5.2%	8.2%	13.0%

ever, differed as much as 10 per cent in weight. Nitrogen, ash and fat-free dry tissue formed fairly constant proportions of the empty carcass weight, even among different litters and with wide ranges of body weight. This is also true of our series, as shown in table 4, by the coefficients of variability from the means of the constituents determined in check and control rats.

If the data both for the rats analyzed by Mitchell and Carman and for the check and control rats of the present series are plotted as scatter diagrams, there are found to be

very high correlations between body weight or body length and the content of nitrogen, fat-free dry tissue, ash or water, in about the order listed. The constituents least correlated with weight or length are fat and energy. Since initially each trio of rats showed insignificant differences in body weight and length, these high correlations indicate that the discrepancy between estimated and true composition must have been small for all constituents except possibly fat.

As is well known, fat is the most variable constituent of an animal's carcass, and tends to increase with age (Moulton, '23). The coefficients of variation for ether extract in the present series of rats are three to eight times greater than those for any other constituent. As shown in table 5, the control rats at death contained almost twice as much fat as they had initially, although they had gained only 32 per cent

TABLE 5

Summary of total gains and composition of gains in control and treated rats

	BODY LENGTH, CM.	LIVE BODY WEIGHT, GM.	EMPTY CARCASS WEIGHT, GM.	COMPOSITION OF EMPTY CARCASS WEIGHT GAIN, GRAMS AND PER CENT					ENERGY, CAL.
				Water	Ether ext.	Fat-free dry tissue	Total N.	Ash	
12 controls									
Total gain	18.2	764	716	324	281	111	15.4	22.7	3161
Per cent of initial datum	7.4	32.1	31.3	22.8	96.7	19.2	20.7	23.1	59.3
Per cent of gain in E.C.W.				45.2	39.3	15.5	2.15	3.16	4.41[1]
12 treated									
Total gain	33.1	1295	1217	771	162	284	37.9	45.1	2820
Per cent of initial datum	13.5	54.9	53.7	54.9	56.3	49.6	51.5	46.4	53.5
Per cent of gain in E.C.W.				63.3	13.3	23.4	3.12	3.71	2.32[1]
Excess gain of treated	14.9	531	501	447	−119	173	22.5	22.4	−341
Per cent of initial datum	6.1	22.5	22.1	31.8	−41.5	30.2	30.6	23.0	−6.5
Per cent of excess E.C.W. gain				89.2	−23.8	34.5	4.49	4.47	−0.68[1]

[1] Calories per gram of empty carcass weight.

of their initial weight. The percentage of water tended to decrease with increase in fat. The energy content reflects the changes in fat and was the second most variable constituent.

Total nitrogen formed a remarkably constant proportion of the fat-free dry tissue, ranging from 125 to 132 mg. per gram for all three groups of check, control and treated animals. The nitrogen content per gram of fat-ash-free dry tissue ranged from 15.1 to 16.3 per cent for the whole group of thirty-two check, control and treated animals analyzed. The mean was 15.6 per cent with a standard deviation of ± 0.33. This is slightly lower than the commonly accepted value of 16 per cent, probably because the ash values are somewhat low.

The water content closely paralleled the nitrogen content of the carcass. The ratios of water to total nitrogen were: check rats, 19.1; controls, 19.4; treated, 19.5; gain of controls, 21.0; gain of treated, 20.4. The ratios of ash to total nitrogen were also fairly constant: checks, 1.31; controls, 1.34; treated, 1.28; gain of controls, 1.47; gain of treated, 1.19.

The most striking finding as to composition was the fact that the treated rats retained almost exactly their initial average percentage composition for all constituents and for energy per gram of empty carcass weight. All of the differences were less than their probable errors, except the difference in the percentage of ash, which was only twice its probable error. As this would indicate and as shown in table 5, the gain by the treated rats for every constituent was in the same proportion as the gain in empty carcass weight—around 50 per cent of the initial datum. This means, too, that the composition of the gains in weight as given in table 5, was closely similar to the composition of the rats themselves. The controls, on the other hand, showed the characteristic changes with age that would be expected in well-nourished rats after puberty and reaching the plateaus of their growth curves—relative decreases in the proportions of water, nitrogen and fat-free dry tissue, and increases in fat and energy. These differences were, respectively, 5.6, 4.9, 6.2, 7.3 and 7.0 times

their probable errors, which indicates significance notwithstanding the slight uncertainty as to the estimated initial composition. The decrease in the percentage of ash was only 2.5 times the probable error of the difference and is perhaps not significant.

The differences in percentage composition between the control and treated rats at the end had the following ratios to their probable errors: water, 6.1; fat, 6.6; nitrogen, 4.0; ash, 0.7; fat-free dry tissue, 5.8 and energy, 6.6. These ratios indicate high significance for all differences except ash, and are based entirely upon analyzed compositions. The number

TABLE 6

Statistical analysis of excess gains of twelve treated over twelve control rats

VARIABLE	MEAN EXCESS GAIN OF TREATED GROUP	t (FISHER)	P
Body length	1.24 cm.	7.18	< 0.01
Live weight	44.25 gm.	5.92	< 0.01
Empty carcass weight	41.75 gm.	6.65	< 0.01
Fat-ash-free dry tissue	12.58 gm.	7.86	< 0.01
Total nitrogen	1.88 gm.	7.81	< 0.01
Water	37.25 gm.	5.67	< 0.01
Ash	1.87 gm.	5.23	< 0.01
Ether extract	−9.98 gm.	−2.74	< 0.02
Total energy	−28.45 cal.	−0.99	< 0.35

of animals in each group was small, however. Since the true value of the standard deviation may only be roughly approximated in small samples, a more rigorous method of statistical treatment would be the determination of 't' and P values for Fisher's ('30, pp. 107 and 139) measure of significance for the difference in the means of small samples. These values for the difference in mean gains of treated and control groups, for the several variables are given in table 6. The probability values (P) indicate high degrees of significance except for total energy. The differences in percentage composition of control and check rats, analyzed by the same method, are all highly significant except for the percentage of ash. The differences in the percentage composition of the

treated and the check rats are entirely insignificant for all variables.

Nitrogen and ash balances. The nitrogen and ash balances, as determined in eight pairs, are shown in table 7. Of the total nitrogen intake, 3.5 per cent for the controls and 4.8 per cent for the treated was apparently lost in collection, perhaps as ammonia from the urine. For ash, on the contrary, more was recovered in urine, feces, and carcass gain than was consumed. This is in part accounted for by the fact that the acid used in washing the funnels and brass screens dissolved some metal. The retentions of both nitrogen and

TABLE 7

Summary of nitrogen and ash balances

GROUP	INTAKE	EXCRETION AND WASTAGE				RETENTION BALANCE	RETENTION, TISSUE ANALYSIS	DIFFERENCE
		Urine	Feces	Shed hair	Total			
I. Nitrogen								
(A) 8 controls								
Grams	234.8	185.3	31.1	1.8	218.3	16.5	8.3	8.2
Per cent of intake		78.9	13.3	0.8	93.0		3.5	3.5
(B) 8 treated								
Grams	234.3	171.6	25.3	2.7	199.6	34.7	23.5	11.2
Per cent of intake		73.2	10.8	1.2	85.2		10.0	4.8
Difference (B–A)								
Grams		−13.7	−5.8	0.9	−18.7	18.2	15.2	3.0
II. Ash								
(A) 8 controls								
Grams	405.6	202.6	198.0	2.5	403.0	2.6	13.7	11.1
Per cent of intake		50.0	48.8	0.6	99.4		3.4	2.7
(B) 8 treated								
Grams	405.0	197.0	186.0	3.1	386.2	18.8	28.7	9.9
Per cent of intake		48.6	45.9	0.8	95.4		7.1	2.4
Difference (B–A)								
Grams		−5.6	−12.0	0.6	−16.8	16.2	15.0	−1.2

ash, determined directly by carcass analysis, are far more reliable than the retentions determined indirectly from the balance between intake, excretion and wastage. The excess retentions by the treated rats, however, show close agreement by the direct and indirect methods of determination, indicating that the errors of collection were approximately the same for the two groups.

The weekly excess urinary nitrogen excretions by the eight controls over their treated mates for the first 8 weeks of the experiment were serially: 4.2, 3.8, 0.2, 2.7, 0.9, 1.5, 0.9 and −1.1 gm. The weekly excess retention by the treated rats thus paralleled their excess gains in weight, as shown in table 1. The treated rats also had less nitrogen and less ash in their feces than had the controls. Both the weights of the dry feces and the percentage content of nitrogen and ash were slightly less. Whether the differences indicate better digestibility of the food or decreases in the 'metabolic' nitrogen and ash of the feces was not determined.

As compared with the eight animals in table 6, the whole group of twelve controls retained 4.3 per cent of the nitrogen, and 3.6 per cent of the ash consumed in their food, according to the carcass analyses. The twelve treated rats retained in their tissues 10.5 per cent of the nitrogen and 7.3 per cent of the ash of their food.

Energy balance. The twelve treated rats retained 6.9 per cent and the twelve controls 7.7 per cent of their total energy intake of 41,019 Calories. Thus, although the empty carcasses of the treated rats weighed 501 gm. more than those of the controls, their total energy content was 400 Calories, or 0.5 per cent, less. This was of course due to the greater fat content of the controls. The difference in percentage retention is not significant statistically because of the large individual variations in energy content. The metabolizable portion of the energy intake for the controls amounted to 34,412 Calories, or 83.9 per cent, and for the treated 34,791 Calories, or 84.8 per cent. The metabolizable portions were determined in the eight pairs used for urine and feces collection by sub-

tracting the non-utilizable energy of the feces, urine, shed hair and alimentary tract contents. All of these items were determined in the oxycalorimeter except the calorific value of the urine, which was assumed to be 8.09 Calories per gram of nitrogen.

The summated surface area for the controls for the 763 days of the experiment was 25.34 sq.m. and for the treated rats 27.17 sq.m. The average basal metabolic rates for normal rats of the same stock in over 100 determinations are 760 Cal. per day per square meter for females and 810 Cal. for males. The basal metabolic requirement for the controls for the whole period was then 19,680 Calories, or 57 per cent, of the metabolizable energy intake. They stored 3,161 Calories, or 9 per cent. Thus 66 per cent of the total metabolizable energy is accounted for, leaving 34 per cent for muscular activity and specific dynamic action. Similar figures can be approximated with somewhat less assurance for the treated rats. On the assumption that their average basal metabolic rates were the same as the controls, a total of 69 per cent of the metabolizable energy intake could be accounted for by the basal metabolic requirements and the energy stored in the carcasses. The energy used for muscular exercise and for specific dynamic action would then have been slightly less than in the controls. We have never found increases in the basal metabolic rate in rats as a result of treatment with the growth hormone, but on the contrary, have found variable decreases in the heat production per square meter of body surface (Lee and Gagnon, '30). These decreases ranged from 0 to 39 per cent and averaged about 16 per cent. If this average decrease is assumed to have occurred in the treated rats, then the portion of their total utilizable energy intake accounted for in basal metabolic requirements and in retention by the tissues was 59 per cent, leaving 41 per cent for muscular activity and specific dynamic action. The impression which was had of the somewhat greater activity of the treated animals would support these assumptions, but further objective evidence under similar conditions is necessary.

The energy cost per gram of weight gained showed striking differences in the two groups. For each gram of empty carcass weight gained the twelve controls expended 43.7 Calories, the twelve treated 26.3 Calories and the two treated, ad libitum fed rats 23.1 Calories. For each gram of gain in protein ($N \times 6.25$) the controls used 325 Calories, the treated 135 Calories and the ad libitum fed, 121 Calories.

It is of incidental interest to record the total heat production of the rats of this series under the environmental conditions which obtained, i.e., individual confinement in small cages without nest material and at a temperature of 25° to 28°C. This can be done with considerable accuracy since both the metabolizable portion of the food energy consumed and the heat value of the materials retained in the carcasses are known. The heat production per day and per square meter of body surface was: control females, 1208 Calories; control males, 1285 Calories; treated females, 1134 Calories; treated males, 1273 Calories.

Ad libitum feeders. The two treated rats given food ad libitum showed qualitatively the same responses to treatment as did their treated mates of pairs 2 and 3 on limited food intake. Their combined empty carcass weight at the end of the experiment was 527 gm., and its composition was: water, 61.5 per cent; ether extract, 12.8 per cent; fat-free dry tissue, 25.7 per cent; nitrogen, 3.3 per cent; ash, 4.4 per cent, and energy 2.4 Calories per gram. The composition of the gains is given in table 8. Their excess gains were remarkably larger in amount than those of the treated mates on paired-feeding when it is considered that their total food consumption was only 13 per cent greater. They gained 66 per cent of their initial live weight, their two control mates gained 23 per cent, and their two treated mates on paired-feeding gained 47 per cent. Likewise, the percentage retentions in the tissues of the nitrogen and ash of the food consumed showed marked differences. For nitrogen, the values were: 3.0 per cent for the controls, 7.9 per cent for the two treated on paired-feeding and 11.4 per cent for the two treated fed ad libitum. For ash the values were 2.3, 4.5 and 8.1 per cent, respectively.

The findings of Bierring and Nielson ('32) on the composition of growth hormone-treated rats under ad libitum feeding are in accord with our data on these two comparable animals.

TABLE 8

Composition of gains in two control, two treated and two treated ad libitum fed rats

GROUP	EMPTY CARCASS WEIGHT	COMPOSITION OF EMPTY CARCASS GAIN					ENERGY, CAL.
		Water	Ether ext.	Fat-free dry tissue	Nitrogen	Ash	
2 controls							
Gain, grams	76.4	24.4	44.8	7.1	1.5	2.1	457
Per cent of initial datum	24.1	12.2	152.4	8.2	14.0	13.3	69
2 treated							
Gain, grams	154.0	70.3	56.2	27.5	4.0	4.0	655
Per cent of initial datum	47.7	34.4	188.0	31.2	36.3	25.5	97
2 treated, food ad lib.							
Gain, grams	215.5	126.6	38.8	50.0	6.6	8.1	593
Per cent of initial datum	69.0	63.9	134.3	58.6	61.7	53.1	91

DISCUSSION

The fact that on the same food intake the treated rats were able to show a considerable excess gain in weight, and the fact that the gain differed markedly in composition from the gain made by their control mates are strong evidence that metabolic processes were affected by the hormone. The data also give clear evidence that the growth produced has the same characteristics as the growth exhibited by young animals after puberty and before reaching their growth plateaus. Among these characteristics are the marked retention of nitrogen, the synthesis and deposition of protein and the relatively high content of water and low content of fat. The hormone apparently causes the retention of, or the return to, those metabolic characteristics which are possessed by young animals rapidly gaining in weight.

To define more precisely the specific effect of the hormone in bringing about these metabolic changes is difficult from the data of a study of this sort. All of the variables determined, except possibly fat, are closely interrelated. This is particularly shown by the constancy of the ratios of water to nitrogen or ash in all three groups of animals and in the gains of the treated and control animals. Consequently, it is not to be expected that a sharp separation of specific effects can be made by a statistical analysis of the data, although such analysis is valuable for confirmation or denial of the inferences from other considerations. The values of t in table 6, for example, are strictly only statistical constants from which may be derived the probabilities of the significance of the differences between the two groups. The order of magnitude of the t values, however, supports the inference that the constituents most specifically influenced by the hormone were nitrogen and fat-ash-free dry tissue. The increases in water and ash in the treated animals might well have been secondary effects of the increase in protein. The increased hydration observed could have occurred independently of any change in protein or fat content, but it is inconceivable that an excess gain in nitrogenous constituents (outside of hair and epidermal structures), amounting to 22.5 gm. of nitrogen could have occurred without the simultaneous retention of a considerable amount of water. A portion of the excess gain in ash can similarly be ascribed to the requirements of the added tissues, and another portion to deposition as bone.

The treated animals necessarily obtained a smaller proportion of their energy requirements from the oxidation of nitrogenous food constituents than did their controls, since they retained about 140 gm. more of protein ($N \times 6.25$). They must also have oxidized more fat than the controls, since they gained about 120 gm. less and since the total fat gain for either group was less than the fat intake. Either a specific effect of the hormone on the oxidation, synthesis or storage of fat, or the necessity of obtaining more of their energy expenditure from fat combustion, may account for the lower

fat content of the treated animals. Gaebler ('33) has reported that in dogs administration of the growth hormone causes a lowering of the R. Q., indicating an increased oxidation of fat.

It is apparent from the distributions shown in table 1 that the excess gain of the treated rats was greatest for the first week and declined progressively thereafter. This would be expected if there was induced in the animals a more rapid growth rate which was still limited by the same general factors conditioning the preexisting rate. There is no reason for assuming that the weight increase at first was composed chiefly of water or was not true growth. The excess nitrogen retention of the treated over the control group, as judged by urinary excretion, was greater for the first than for any subsequent week. The excess weight gain for the first week could thus all be accounted for as true body growth with even a lower ratio of water to nitrogen than that of the carcass itself.

The decline in the rate of excess gain might be considered to have been due to an increasing partial inanition if there had been also a progressive decline in the proportion of the food energy available for growth, after meeting the requirements for maintenance, specific dynamic action and muscular activity. The surface area, summated by weeks for the whole period, was only 8 per cent greater for the treated than for the controls. If the basal metabolic rates were not increased, the corresponding change in maintenance requirements is not of sufficient magnitude to be an important factor. Specific dynamic action is apparently slight in the rat (Mitchell and Carman, '26). The requirement for muscular energy, both because of an increase in the activity and the greater weight of the animals, was apparently somewhat increased.

It is doubtful, however, that the available energy alone could have been the only or even the most important limiting factor for the decrease in the rate of excess gain. The total amounts of food consumed by each group, serially for the first 8 weeks, were: 874, 1110, 1087, 1146, 1143, 1099, 1045 and 1065

gm. Thus, for the first week in which the treated rats made their greatest excess gain, the food consumption was 16 to 24 per cent less than for any subsequent week. The response of the two treated rats on ad libitum feeding offers further evidence. Their excess gain in weight over their two controls was 79 per cent greater than that of their two treated mates on paired-feeding, with a food consumption only 13 per cent greater. They also showed the same progressive decrease in the rate of excess gain from week to week. Their excess nitrogen retention, from urine analysis, also paralled their excess gain in weight.

The impermanence of the gains in weight when administration of growth extracts is discontinued should be mentioned. After the end of a long period of such treatment there is usually a loss within a week or so of upward of one-fourth of the excess gains, and the growth curve thereafter remains fairly level for some time. After short periods of treatment the loss of the excess gains may be much greater, especially in hypophysectomized rats. For several years we have used for assay purposes 3- or 4-day periods of treatment in groups of about fifteen animals. The average losses in weight 96 hours after the last of three daily injections of potent extracts were calculated for eighty-eight such assays using over 200 rats and varying effective dosages. The loss for hypophysectomized rats amounted to 67 per cent and for normal adult females to 50 per cent of the weight gained in the preceding 72 hours.

A year previous to making the experiments which this paper describes in detail, a similar paired-feeding test was made with nine pairs of rats. It had to be ended after the third week because the supply of potent purified extract became exhausted and we were unable to prepare again a suitable extract with which to continue. The results for the short time were as striking as those from the present more complete experiment. At the end of the third week the treated rats had gained 248 gm. more than their litter-mate controls and had excreted 5.7 gm. less of nitrogen in the urine. From

the third to the fifth weeks (without extract) the treated group lost 50 gm. in weight and excreted 4.5 gm. of nitrogen more than their controls. Their weight then remained fairly constant until the ninth and tenth week when they began to show definite gains again. At the end of the tenth week, still under paired-feeding the controls had just caught up with them in weight, and had retained 1.8 gm. more of nitrogen.

SUMMARY

The effect of the growth hormone of the anterior pituitary upon the gain in weight and upon the composition of the gain was determined in rats under a paired-feeding regimen. Twelve pairs, each composed of litter-mates of the same sex, initial body weight and length were used. The food consumption in each pair was kept the same for control and treated members. After 8 to 11 weeks the animals were killed and their carcasses analyzed. Their compositions at the beginning of paired-feeding and treatment were estimated from the analyses of other litter-mates killed at that time.

The twelve controls showed the characteristic changes in body composition with age that would be expected. These changes were statistically significant decreases in the proportion of water, nitrogen, fat-free dry tissue, and ash, and increases in the percentage of fat and in the heat value of the tissue. They gained a total of 764 gm. in body weight and 18.2 cm. in length.

The twelve treated animals retained almost exactly their initial composition in all constituents and in the heat value of their tissue. Their excess gains over their control mates, on the same food intake, were: live weight, 531 gm; body length, 15 cm.; water, 447 gm.; fat-free dry tissue, 173 gm.; nitrogen, 22.5 gm.; ash, 22.4 gm.; fat, – 119 gm.; energy, – 341 calories. The composition of their gains thus differed markedly from that of the controls, but was closely similar to the initial composition of the animals themselves.

The nitrogen and ash balances determined indirectly by analyses of food, urine and feces corroborate the direct find-

ings from carcass analysis. The weekly excess nitrogen retention in the treated rats paralleled closely the weekly excess gain in weight, and indicates that even the excess gain in weight for the first week constituted true growth.

The energy expenditure was 1.7 times as great for the controls as for the treated animals per gram of weight gained, and 2.4 times as great per gram of protein gained. The treated animals burned more fat and less protein than did the controls.

Two treated animals on ad libitum feeding gave responses the same qualitatively, but greater in amount than did their treated mates on paired-feeding.

Statistical analysis of the data indicates that nitrogen and fat-ash-free dry tissues were the constituents most specifically influenced by the hormone.

We are indebted to Dr. T. M. Carpenter and to Dr. F. G. Benedict for their generous criticism, and to Mr. E. M. Jellinek for assistance in the statistical treatment of the data.

LITERATURE CITED

ANDERSON, W. E., AND A. H. SMITH 1932 Further observations of rapid growth of albino rat. Am. J. Physiol., vol. 100, p. 511.

BENEDICT, F. G., AND E. L. FOX 1925 A method for the determination of the energy values of food and excreta. J. Biol. Chem., vol. 66, p. 783.

BENEDICT, F. G., AND R. D. MILNER 1907 Experiments on the metabolism of matter and energy in the human body 1903–1904. U. S. Dept. Agri. Office of Exp. Stations, Bulletin 175, p. 144.

BIERRING, E., AND E. NIELSEN 1932 Composition of tissues of albino rats treated with alkaline pituitary extracts. Biochem. J., vol. 26, p. 1015.

DONALDSON, H. H. 1924 The rat. Memoirs of The Wistar Institute of Anatomy and Biology, no. 6.

EVANS, H. M., AND M. E. SIMPSON 1931 Hormones of anterior hypophysis. Am. J. Physiol., vol. 98, p. 511.

FISHER, R. A. 1930 Statistical methods for research workers. 3rd ed. Oliver & Boyd, London.

GAEBLER, O. H. 1933 Further studies of anterior pituitary extracts. J. Biol. Chem., vol. 100, p. xlvi.

LEE, M. O., AND E. CLARK 1929 Determination of the surface area of the white rat with its application to the expression of metabolic results. Am. J. Physiol., vol. 89, p. 24.

LEE, M. O., AND J. GAGNON 1930 The effects of growth promoting and gonad stimulating principles of the anterior lobe of the pituitary on basal gaseous metabolism in the rat. Endocrinology, vol. 14, p. 233.

MITCHELL, H. H., AND J. R. BEADLES 1930 Paired-feeding method in nutrition experiments and its application to problem of cystine deficiencies in food proteins. J. Nutrition, vol. 2, p. 225.

MITCHELL, H. H., AND G. C. CARMAN 1926 The composition of the gains in weight and the utilization of food energy in growing rats. Am. J. Physiol., vol. 76, p. 398.

MOULTON, C. R. 1923 Age and chemical development in mammals. J. Biol. Chem., vol. 57, p. 79.

RIDDLE, O., R. W. BATES, AND S. W. DYKSHORN 1932 New hormone of anterior pituitary. Proc. Soc. Exper. Biol. and Med., vol. 29, p. 1211.

EDITORIAL

TRANSFER OF THE JOURNAL OF NUTRITION TO THE WISTAR INSTITUTE

The first suggestion of a journal in America, devoted entirely to the subject of nutrition, came from Mr. Charles C. Thomas to the writer in the fall of 1918. After consulting several of his colleagues, the writer was obliged to report that the time did not appear to be opportune for such a venture. Nearly 10 years later, after Mr. Thomas had inaugurated a publishing business of his own, the proposal was renewed. This time consultation with several colleagues brought a different response, and in a few weeks a nucleus of five members of an editorial board was assembled and it was decided to proceed with plans for such a publication, Mr. Thomas to be publisher, and other persons, upon whom the existing group could agree unanimously, were to be added to the board until a number of approximately a dozen was reached.

The ownership of The Journal of Nutrition from the first was vested in the editorial board, later incorporated under the education law of the State of New York as the American Institute of Nutrition. The instrument of agreement with Mr. Thomas recited definite conditions under which either party might annul the contract by fulfillment of specific terms. No thought was entertained, however, by either party at the time of signing the contract (April, 1928) that the agreement was not to continue in force indefinitely. Relations between the board and publisher proceeded on a perfectly harmonious basis. It was hoped that manuscript support and the number of subscribers would increase sufficiently so that within a couple of years the journal could be published

once a month. The former outran all expectations, but the latter, on account of the economic depression, did not keep pace. The only solution of these difficulties seemed to be to seek a publisher who was not dependent solely upon income from the journal to defray the costs of printing and publishing. The board found such a publisher in The Wistar Institute of Anatomy and Biology, which for nearly 30 years has been supporting the publication of scientific journals and monographs. Negotiations with The Wistar Institute were begun in the spring of 1932 and were continued until terms satisfactory to the three parties in interest, Mr. Thomas, The Wistar Institute and the Board of Editors (who were as yet the only members of the American Institute of Nutrition), were reached, in April, 1933. This agreement called for the transfer of ownership of the journal and its assets at the beginning of 1934 to The Wistar Institute. This transfer was completed formally under date of January 3, 1934.

Ownership is now vested in The Wistar Institute, and the journal will sustain to the American Institute of Nutrition no other relationship than that of official organ.

It is fitting that the Editorial Board and The Wistar Institute should join at this time in an expression of appreciation to Mr. Charles C. Thomas, for his exceptional courtesy and fair attitude in all the recent negotiations and transactions connected with the transfer of the journal. The Managing Editor wishes further to go on record in commendation of the courteous and consistent attention given by Mr. Thomas to the interests of the journal throughout the 5-year period of his association with it, as publisher. Mr. Thomas relinquished his interest in the journal with regret, and yet without hesitancy, when he became convinced that the journal stood a better chance of fulfillment of its aims and purposes in the hands of The Wistar Institute of Anatomy and Biology.

JOHN R. MURLIN.